STEROID HORMONE REGULATION OF VITELLOGENIN GENE EXPRESSION

Author: **David Shapiro**
Department of Biochemistry
University of Illinois
Urbana, Illinois

Referee: John M. Taylor
Department of Microbiology
Hershey Medical Center
Hershey, Pennsylvania

I. INTRODUCTION

Despite remarkable advances in our understanding of the organization of eucaryotic genes, the mechanisms by which eukaryotic gene expression is regulated remain one of the major unresolved questions in biochemistry and molecular biology. The intractability of this problem in spite of intense efforts in numerous laboratories is in large part due to a failure to understand, or even to identify, many of the protein-nucleic acid interactions which lie at the core of differential gene expression. While the enormous technological advances signalled by recombinant DNA technology to isolate and amplify specific nucleic acid sequences, by restriction enzymes and other enzymes which cut, ligate, and act on nucleic acids in a variety of ways and by rapid sequencing techniques for DNA and RNA have made possible a detailed description at the primary sequence level of the structure of a number of regulated eucaryotic genes, a lack of technical innovations of comparable magnitude makes it impossible to even identify the proteins which regulate most developmental processes. Two new techniques, one based on faithful in vitro transcription of eukarytoic genes[1-4] and the other on the use of monoclonal antibodies against single proteins,[5-7] promise new approaches to these problems. While these techniques and eukaryotic gene transfer possess enormous potential, their application to the identification of eukaryotic gene regulatory proteins has barely begun.[3]

Our present inability to identify many of the proteins involved in control of gene expression in development stems in large part from the absence of functional assays for these proteins which are independent of their effects on gene expression. A great deal of attention has therefore focused on systems in which known intercellular signals, especially steroid hormones, control the expression of specific genes. The molecular effects of most hormones are mediated by specific protein receptors capable of stereospecific high-affinity binding of specific hormones. Steroid hormone receptors appear to be bifunctional proteins whose ability to specifically bind labeled hormones makes them identifiable biochemically, while their ability to act at the chromatin level allows them to control the expression of specific genes. While several biochemical systems responsive to steroid hormones have received considerable attention,[8,9] the induction by estrogens of the egg white protein ovalbumin in the magnum portion of the chick oviduct remains the most widely studied model for this type of system. In this review, the author will focus on a new model system which is under intensive study in a number of laboratories, the estrogen-mediated induction of the egg yolk precursor protein, vitellogenin, in amphibian and avian systems. The focus will be primarily on vertebrate systems as more detailed information concerning gene structure and expression is available in these systems than in the very interesting juvenile hormone

regulated insect systems. Excellent reviews of the insect vitellogenins have appeared recently.[10,11]

II. VITELLOGENIN

The formation of egg yolk proteins in oviparous vertebrates such as *Xenopus laevis* and the hen, *Gallus gallus,* involves synthesis of a yolk protein precursor by the liver, secretion into the serum and uptake and processing by the ovary.[12-20] The vitellogenin monomer is a calcium binding phospholipoglycoprotein with a monomer molecular weight variously estimated at 190,000 to 220,000 in *Xenopus*[12,15,21,22] and 210,000 to 230,000 in the chicken.[20,23] The secreted form of vitellogenin isolated from serum is a dimer and it is not clear whether or not the three distinct vitellogenin polypeptide chains which have been identified[24] form heterodimers in vivo. Vitellogenin is selectively removed from serum by the ovary through an uptake mechanism which has not been extensively characterized at the molecular level. The vitellogenin monomer is cleaved in the ovary to yield lipovitellin and phosvitin, an extensively phosphorylated protein which is about 55% serine. Two major forms of *X. laevis* vitellogenin have been identified[25] and the following tentative scheme for their organization has been advanced:[25] (1) NH_2-lipovitellin I -phosvitin-lipovitellin II -COOH, and (2) NH_2-lipovitellin I -[phosvette I, phosvette II]- lipovitellin II -COOH. (It is proposed that phosvettes arise through cleavage at an additional site in the serine-rich portion of each vitellogenin monomer.) Two forms of avian vitellogenin have been detected[26] and they appear to contain two molecules of phosvitin and one molecule of lipovitellin.[20] The relation between the various *Xenopus* vitellogenin polypeptides and the four different vitellogenin genes (see below) remains to be established. The site of vitellogenin synthesis and the mechanisms of secretion, uptake, and processing have been studied for many years, and are treated in several reviews.[27-32]

III. INDUCTION OF VITELLOGENIN SYNTHESIS AND VITELLOGENIN mRNA

Administration of estradiol-17β to male *X. laevis* or to cockerels evokes the massive and prolonged synthesis of vitellogenin and its cognate mRNA. Vitellogenin synthesis can account for as much as 70% of liver cell mRNA and protein synthesis in *Xenopus*[22,33] (Figure 1) and 10 to 40% of liver cell mRNA and protein synthesis in different strains of rooster.[34,35] The induction of vitellogenin synthesis in *X. laevis* can occur without cell differentiation or cell death.[36] Although significant cellular hyperplasia occurs in avian systems it does not appear to play a major role in the induction of vitellogenin synthesis.[19,34] Vitellogenin synthesis can be induced in both organ cultures and in primary liver cell monolayers derived from amphibians.[36-40] The maintenance and induction of vitellogenin synthesis in avian and insect cells has proven quite difficult and only modest success has been reported.[10,11]

The induction of vertebrate vitellogenin represents a relatively simple system which is readily amenable to biochemical analysis. Vitellogenin gene expression is induced by well-defined biochemical signals (steroid hormones). The specificity of the hormonal response is very high as only estradiol-17β and diethylstilbestrol are capable of efficient induction of vitellogenin mRNA.[41] The induction of vitellogenin is reversible, occurs in a single cell type, and can be achieved without cell division or cell death. Induction of vitellogenin results in production of large amounts of protein and mRNA which are easy to isolate due to their unusual size and abundance. Induction can be achieved in vitro in a chemically defined environment. A major drawback of this system is that it has not been

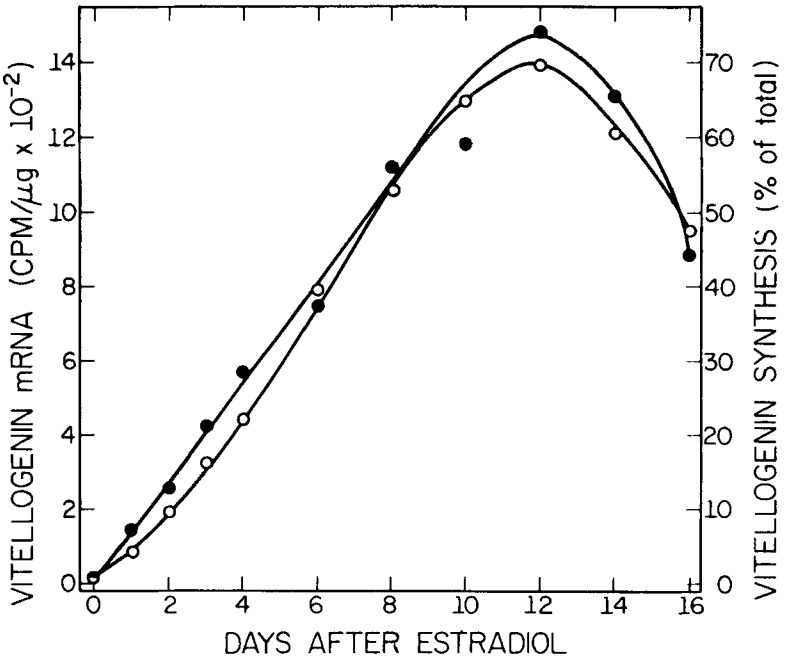

FIGURE 1. Time course of estrogen induction of vitellogenin mRNA and vitellogenin synthesis. Male *X. laevis* received injections of estradiol-17β on day 0. The fraction of liver protein synthesis devoted to the synthesis of vitellogenin was determined by comparing the level of immunoprecipitable vitellogenin to the level of trichloracetic acid precipitable protein (O——O). The ability of extracted RNA to code for immunoprecipitable vitellogenin in a rabbit reticulocyte lysate protein synthesizing system was determined. Results are plotted as vitellogenin synthesis per μg of RNA (●——●). (From Shapiro, D. J., Baker, H. J., and Stitt, D. T., *J. Biol. Chem.*, 251, 3105, 1976. With permission.)

possible so far to make use of modern genetic techniques in the study of vertebrate vitellogenin synthesis.

The large size (approximately 30S) and high abundance of vertebrate vitellogenin mRNA facilitated its isolation.[34,42-44] In experiments performed at approximately the same time in several laboratories cDNA hybridization probes synthesized from purified vitellogenin mRNA templates were used to quantitate the induction of vitellogenin mRNA.[45-47] Vitellogenin mRNA was undetectable in the livers of unstimulated male *Xenopus laevis* in hybridization experiments with a limit of detection of less than one molecule of vitellogenin mRNA per cell.[42,45,58] In roosters, a low level of vitellogenin mRNA corresponding to 1 to 5 molecules per cell was detected.[34] The time course of accumulation of vitellogenin mRNA in *Xenopus* liver cells following primary estrogen stimulation appears to be dose dependent. Using large pharmacologic doses of estrogen to maximize the vitellogenic response, Baker and Shapiro detected vitellogenin mRNA within 4½ hr of initial estrogen treatment.[45,48] Ryffel et al.[46] who employed much smaller doses of hormone did not observe cytoplasmic vitellogenin mRNA until 9 to 12 hr after primary estrogen stimulation. The rate of accumulation of *Xenopus* liver vitellogenin mRNA increases between 4½ and 24 hr after primary estrogen stimulation and is linear for several days thereafter.[45,46,48] The observed level of vitellogenin mRNA in fully induced liver cells and the peak mRNA level obtained are also dependent on estrogen dose. At low but still nonphysiologic levels of estradiol-17β vitellogenin mRNA

peaks at approximately 10% of cellular mRNA 6 days after primary estrogen stimulation,[46] while at higher levels of hormone vitellogenin mRNA levels peak approximately 12 days after estrogen stimulation and comprise as much as 70% of mRNA in liver cells.[22,33]

In the absence of additional exogenous estrogen the cellular estradiol-17β concentration declines and ultimately returns to basal levels. The withdrawal and subsequent disappearance of estrogen is accompanied by a profound decline in cellular vitellogenin mRNA levels, which reach a level of less than one molecule per cell approximately 60 days after primary estrogen stimulation.[45,48] Restimulation with estrogen at that time (secondary stimulation) elicits a rapid appearance of new vitellogenin mRNA and a more rapid accumulation of vitellogenin mRNA throughout secondary estrogen stimulation (Figure 2). The enhanced secondary response to estrogen in *X. laevis* has been observed at both the protein and mRNA levels.[30,36,45,46,48]

The induction of vitellogenin mRNA in the rooster follows a similar but more rapid time course. Avian vitellogenin mRNA begins to accumulate approximately 30 min after estrogen administration following both primary and secondary estrogen stimulation. The rate of vitellogenin mRNA accumulation is 6- to 7-fold greater during early secondary estrogen stimulation.[47,49] The elucidation of the molecular mechanisms which account for the enhanced secondary vitellogenic response observed in amphibian and avian systems represents one major area of current work. With this outline of the induction of vitellogenin it is possible to focus on recent work which attempts to define both the structure of vitellogenin genes and the individual steps in the induction process and their role in the regulatory system.

IV. ESTROGEN RECEPTORS AND VITELLOGENIN GENE EXPRESSION

A. Level of Estrogen Receptor During Vitellogenin Induction and Withdrawal

In the general model for steroid hormone effects on gene expression the cytoplasm of target cells contains high levels of protein(s) capable of stereospecific high affinity hormone binding.[8,50,51] The binding of hormone to the receptor results in "transformation" of the receptor to a form with a high affinity for nuclear components. The receptor then translocates into the nucleus, binds to chromatin and exerts its nuclear effects. The estrogen receptor systems involved in induction of vitellogenin deviate from this model in several important respects. Westley and Knowland[52] first identified a high affinity ($K_d = 4\text{-}5 \cdot 10^{-10}$ M) estradiol-17β binding protein in *Xenopus* liver cytosol. The high affinity for estradiol-17β and the specificity of binding led them to suggest that this protein was the estrogen receptor involved in vitellogenin synthesis. They reported a roughly equal distribution of receptor with approximately 100 high affinity estrogen binding sites in both the nucleus and cytoplasm of unstimulated cells. In contrast, Hayward et al.[53] recently observed several-fold higher levels of receptor in both the nucleus and the cytoplasm from unstimulated *X. laevis* liver cells. The differences in observed nuclear receptor levels (550 sites/cell[53] vs. 100 sites/cell[52]) may be due to the fact that Hayward et al. employed a rather crude nuclear preparation while Westley and Knowland employed a nuclear preparation which was washed and resedimented several times. It is possible that substantial leakage of estrogen receptor from the nucleus occurs during the course of nuclear isolation. It is improbable that the high level of estrogen receptor observed in nuclei from unstimulated liver cells is due to nonspecific trapping of cytoplasmic receptor during nuclear isolation — as nuclei from unstimulated and withdrawn *Xenopus* liver cells contain similar levels of nuclear receptor but differ by at least five-fold in levels of cytoplasmic receptor. The high level of nuclear receptor in unstimulated cells might be explicable in terms of low level expression of the

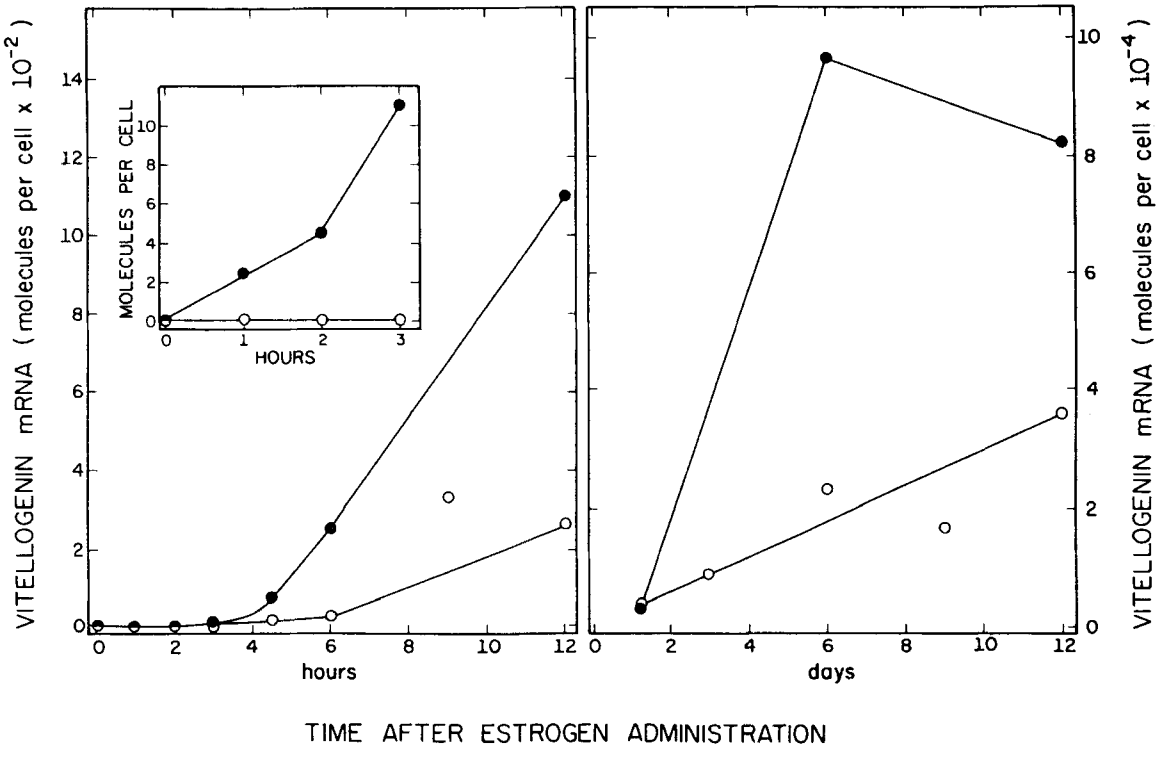

FIGURE 2. Comparison of vitellogenin mRNA sequence accumulation during primary and secondary estrogen stimulation. Male *X. laevis* were either unstimulated (primary stimulation; [○——○]) or injected one time with estrogen and withdrawn for 60 to 65 days (secondary stimulation [●——●]). All animals received estrogen at 0 hours. Liver RNA was isolated at the indicated times and hybridized in RNA excess to vitellogenin cDNA. The measured value of $Crt_{1/2}$, the yield of RNA per gram of liver, the size of vitellogenin mRNA and the number of hepatocytes per gram of liver were used to calculate the number of molecules of vitellogenin mRNA per cell. The inset in the left panel illustrates the rapid onset of vitellogenin mRNA accumulation during the first few hours of secondary (but not primary) stimulation. The left panel illustrates that accumulation of vitellogenin mRNA is several times more rapid in early secondary stimulation than in early primary stimulation. The overall time course of induction shown in the right panel demonstrates that the peak induction of vitellogenin mRNA is reached more rapidly and is greater in secondary estrogen stimulation than in primary stimulation. (From Baker, H. J. and Shapiro, D. J., *J. Biol. Chem.*, 253, 4521, 1978. With permission.)

vitellogenin genes in these cells. However, as indicated below, unstimulated *Xenopus* liver cells do not contain detectable vitellogenin mRNA[42,45] and do not appear to transcribe the vitellogenin genes which are in an inactive DNase I insensitive conformation in these cells.[54,55] It is not known whether the nuclear estrogen receptor in unstimulated cells is bound at specific high affinity sites in chromatin as the thyroid hormone receptor appears to be, or is more loosely and randomly associated with the chromatin.

Administration of estradiol-17β results in the induction of nuclear receptor which reaches a level of approximately 2000 sites per cell.[53] The cytoplasmic receptor level in estrogen stimulated cells varies in a time-dependent fashion and is generally less than 1000 sites per cell.[53]

Administration of estrogen to male *X. laevis* previously induced to synthesize vitellogenin mRNA, but inactive in vitellogenin mRNA synthesis at the time of restimulation with estrogen, results in more rapid activation of the vitellogenin genes and accelerated accumulation of vitellogenin mRNA (see Figure 2). Measurement of estrogen receptor levels in these withdrawn animals revealed that the nuclear receptor which remains elevated for an extended period[56] has returned to the basal level prevailing in unstimulated cells.[53] In constrast the cytoplasmic receptor level increased many-fold to several thousand sites per cell. The rapid apparent turnover of nuclear receptor[56] and the long duration of the effect suggest that the cytosol receptor detected has been synthesized in the cytoplasm and has never entered the nucleus as opposed to return of nuclear receptor to the cytoplasm following estrogen withdrawal. The shift in the nuclear:cytoplasmic receptor ratio from approximately 1:1 in unstimulated cells to 1:3 in withdrawn cells has not been observed in other systems responsive to steroid hormones. This large pool of cytoplasmic receptor may be rapidly translocated into the nucleus following estrogen stimulation, and thereby facilitate the rapid induction of vitellogenin mRNA. Since no comparable pool of cytoplasmic receptor is detected in unstimulated cells, efficient induction of vitelloginin mRNA in primary stimulation may require synthesis of additional estrogen receptor. A possible role for this newly discovered pool of cytoplasmic estrogen receptor in the more rapid and efficient expression of the vitellogenin genes during secondary estrogen stimulation of *X. laevis* represents an attractive hypothesis subject to experimental verification.

The estrogen receptor in avian liver is also present at low concentrations. Approximately 300 high affinity estrogen binding sites were detected in unstimulated cockerel cytosol[57] and 150 sites in salt extracts of unstimulated nuclei. Administration of estradiol-17β induces rapid depletion of cytosol receptor, which is slowly replenished. Nuclear receptor levels increase to a peak of approximately 2500 sites per cell 24 hr after estrogen administration at which time cytosol receptor levels were 250 sites per cell.[57] These values are strikingly similar to those we have reported in estrogen stimulated *Xenopus* liver.[53]

B. Properties of the Estrogen Receptors

The low levels of estrogen receptor in cytosol of unstimulated amphibian and avain liver cells has restricted studies to salt extracted nuclear receptors. The possibility that salt extraction induces receptor aggregation is a serious one and must be assessed through the use of receptor released from nuclei by polyamines or nuclease digestion.[58] The salt extracted nuclear estrogen receptor from *X. laevis* sediments with a molecular weight of approximately 40,000 daltons on sucrose gradients.[52] The short half-life of the *Xenopus* estradiol-17β-receptor complex (210 min at 4°, and 60 to 90 min at 10°) necessitates the use of [^{3}H] estradiol-17β-containing sucrose gradients.[52] The avian estrogen receptor is an asymmetric protein with a high tendency to aggregate. After

trypsinization the molecular weight of the estrogen binding protein of the receptor subunit falls from 56,000 to 40,000 and the higher molecular weight 80,000 and 150,000 dalton aggregates are no longer observed.[58,59,60]

C. The Estrogen Receptor and Vitellogenin Gene Expression

While a relationship between the high affinity estrogen binding proteins identified in *Xenopus* and chicken liver and vitellogenin gene expression has not been conclusively established, the receptor's properties are consistent with a role in vitellogenin synthesis. Only estradiol-17β and diethylstilbestrol exhibit high affinity binding to these proteins[52,53,57] and these are the only hormones which are efficient inducers of vitellogenin mRNA.[36,41,61] These estrogen receptors are the only high affinity ($K_d = 3\text{-}5 \cdot 10^{-10}$ M) estrogen binding proteins detectable in amphibian and avian livers. Administration of estrogen induces both vitellogenin and the nuclear receptor. Nuclear receptor levels peak at approximately the point of maximal vitellogenin mRNA accumulation and the induction of cytoplasmic receptor in withdrawn *Xenopus* is consistent with a role in the enhanced accumulation of vitellogenin mRNA observed early in secondary estrogen stimulation. While this evidence is suggestive rather than conclusive, it seems reasonable to assume that these proteins do in fact represent the estrogen receptors in these tissues.

D. A Receptor Saturation Model of Vitellogenin Gene Expression

In the "classical" model for steroid hormone action estrogen binding to a high-affinity cytosol receptor produces a conformational change in the receptor which is then translocated into the nucleus. In the nucleus the receptor "searches" through the chromatin, binding and dissociating from low-affinity sites, until it binds tightly to the correct high affinity chromatin binding sites.[8,9,50,51] Once bound to the correct site one or two receptors elicit some modification of adjacent chromatin (perhaps through histone acetylation-deacetylation or DNA demethylation). This modification changes the template capacity of the chromatin and is propagated down the chromatin in some fashion. This model is difficult to reconcile with the properties of the *Xenopus* liver estrogen receptor. The *Xenopus* liver estrogen receptor is largely nuclear in unstimulated cells. The vitellogenin genes in these cells are in the transcriptionally inactive DNase I insensitive conformation[54] and the cells do not contain detectable vitellogenin mRNA.[42,46,48]

The author proposes an alternative model for the interaction of hormone receptors with chromatin. In this model the "specific" chromatin regulatory sites have a relatively low affinity for the estrogen-receptor complex and the affinity of these specific sites for E-R complex is only slightly higher than the affinity of a large number of nonspecific sites. Specificity in estrogen action is achieved because the binding of E-R complexes to chromatin is highly cooperative and the small difference in binding energies for individual receptor molecules binding to specific and nonspecific chromatin sites becomes substantial when it is totaled over a substantial number of receptors (perhaps 5 to 10) binding in a cooperative fashion.[62] Full activation (or repression) of a particular gene requires the binding of a large number of receptors. If fewer receptors are bound, a submaximal response is achieved until a critical threshold level of receptors is reached in the nucleus, below which no specific receptor binding can occur[62] and no template activity of the genes is observed. This model, which is based in part on observations of the mode of binding of the T_4 gene 32 protein to nucleic acids,[62] makes several predictions which can be tested experimentally. It explains in functional terms why hormone receptors have the propensity for aggregation so frequently observed and why specific high affinity chromatin binding sites for hormone receptors have proven so elusive. It also explains why administration of steroid hormones frequently produces an induction

of the receptor.[8,9] It provides a simple rationale for the differential effect of E-R on specific genes. For example, the binding of fewer molecules of estrogen receptor might be required to activate synthesis of new receptor RNA than to activate other estrogen regulated genes. The slow rate of vitellogenin mRNA accumulation in early primary stimulation would be explained by a failure to achieve a fully saturating receptor level until more receptor is synthesized. The high level of nuclear receptor in unstimulated *Xenopus* liver cells is compatible with the two possible explanations. The receptor could be randomly distributed in these cells whose nuclei occupy much of the cell volume, or unstimulated male *Xenopus* liver cells may contain levels of estradiol-17β which are sufficiently high to elicit the translocation of the estrogen receptor into the nucleus, but are insufficient to elicit the translocation or synthesis of sufficient estrogen receptor to achieve specific binding and activate transcription of the vitellogenin genes. Current data does not allow for an unequivocal choice between these alternatives. Kinetic studies of [^{3}H]estradiol binding to receptor from unstimulated nuclei which might be expected to distinguish "loaded" from "unloaded" nuclear receptor are complicated by the rapid dissociation of estradiol-17β from the receptor. The available kinetic data suggest that the nuclear estrogen receptor from unstimulated liver cells does not contain bound estradiol-17β[104]

The predominance of cytoplasmic estrogen receptor in unstimulated cockerel liver is similar to the situation prevailing in most steroid responsive systems. However, the level of estrogen receptor in both the amphibian and avian systems is 10- to 100-fold lower than is observed in most other estrogen responsive systems including rat liver and uterus and hen oviduct. The receptor level is so low as to allow a direct test of the hypothesis that estrogen receptors are direct regulators of gene transcription in these systems. If estrogen-receptor is a direct transcriptional activator, much and perhaps most of the receptor should be associated with rapidly transcribed genes as several hundred RNA sequences exhibit large changes in concentration following estrogen administration.[63] Of course, if the receptor saturation model presented above is valid, direct transcription regulation would certainly require binding of most E-R complexes to estrogen responsive genes.

V. STRUCTURE OF THE VITELLOGENIN GENES AND TRANSCRIPTS

X. laevis vitellogenin is coded by a large 6300 to 6500 nucleotide mRNA[42,43] while avian vitellogenin is coded by a 7500 nucleotide mRNA.[34,35] Wahli et al. cloned vitellogenin mRNA by A-T tailing into the ECoRI site of the plasmid pMB9.[64,65] Restriction endonuclease mapping and cross hybridization experiments with vitellogenin cDNA revealed that vitellogenin mRNA was coded by a family of four related genes designated A1, A2, and B1, B2. Within the A and B gene pairs the sequence divergence is about 5% while it is about 20% between each pair of genes.[64-66] The vitellogenin A1 gene is 21 kilobases in length and the A2 gene is 16 kb long. The A1 and A2 genes each contain 33 intervening sequences located at homologous positions within the coding sequences.[66-69] The intervening sequences in the vitellogenin A genes and the 5' flanking region contain large blocks of middle repetitive DNA.[69] The size and structure of the homologous intervening sequences exhibit extensive divergence. The relative stability of the vitellogenin coding sequences and the diversity of the intervening sequences led Wahli et al.[69] to propose that the A1 and A2 genes arose by duplication and are highly conserved by selective pressure. Although the number and location of the intervening sequences in the *Xenopus* vitellogenin A genes is conserved, the DNA in the intervening sequences has undergone extensive change through mechanisms which

appear to include point mutation, deletion, insertion, and possibly duplication. The extensive divergence within the intervening sequences and the occurrence of repeated DNA led to the suggestion that most of the DNA within the intervening sequences may have no specific function.[69]

The chromosomal locations of the four vitellogenin genes are unknown but the failure to isolate overlapping genomic clones indicates that the A1 and A2 genes are at least 30 kb apart.[66] The existence of four related *Xenopus* vitellogenin genes lends support to the theory that the *Xenopus* genome arose through a primitive double duplication. This theory suggests that the four vitellogenin genes lie on at least two different chromosomes rather than in tandem array.

The large mRNA procursor for one of the vitellogenin A genes has been identified and its processing examined.[68] Removal of intervening sequences appears to be an ordered event following multiple pathways. A putative precursor to avian vitellogenin mRNA approximately 22 kb in length has been reported and a cDNA clone of a portion of the avian vitellogenin mRNA molecule has been described.[70-71] The melting characteristics of hybrids between the single avian cDNA clone reported to date and avian vitellogenin mRNA have not been described so no conclusions may be drawn yet concerning the possible existence of multiple genes in this system.

VI. VITELLOGENIN CHROMATIN

The organization of vitellogenin chromatin is a subject of critical importance which is only beginning to be addressed. A knowledge of the properties of estrogen receptors and the structure of the vitellogenin genes is clearly insufficient to establish the mechanism of estrogen regulation. The importance of chromatin structure is graphically illustrated by the fact that chicken liver and oviduct both contain functional estrogen receptor. In oviduct the ovalbumin gene but not the vitellogenin gene is transcribed while in hen liver the vitellogenin gene but not the ovalbumin gene is transcribed.

A. Effect of Estrogen on RNA Polymerase II Levels

In the avian system investigations have centered on the effects of estrogen on the level and activity of RNA polymerase. Titration of RNA polymerase II with [^{3}H]α-amanitin led to the conclusion that an increase in the number of RNA polymerase II molecules and not an increase in their activity[72] is responsible for the two-fold increase in polymerase catalyzed RNA synthesis observed in nuclear extracts[72] and chromatin.[73] Increased activity of RNA polymerase II is one of the earliest estrogen effects in a variety of systems. While this two-fold increase in polymerase activity is undoubtedly of physiologic significance, it is orders of magnitude smaller than the effects of estrogen on synthesis of specific RNAs, such as vitellogenin. In a sense the increase in polymerase activity appears to be a universal and perhaps very primitive response to steroid hormones. Superimposed on this ancient response are the tissue and gene specific effects of steroid hormones.

In addition to RNA polymerase[72] the avian estrogen receptor is enriched two- to four-fold in an actively transcribing chromatin fraction.[74] The estrogen dependent transfer of receptor from a random distribution in chromatin to one in which it is specifically associated with the actively transcribing fraction of chromatin is especially amenable to study in this system.

B. Conformation of Vitellogenin Genes in Chromatin

Chromatin which is undergoing active transcription appears to possess a unique conformation which is reflected in enhanced susceptibility to digestion by DNase I and

by micrococcal nuclease[75-78] and by preferential solubility at low magnesium concentrations.[79] Two proteins which have been implicated in the acquisition of DNase sensitivity have been isolated.[80] The binding of these proteins to DNA may be related to the demethylation of transcriptionally active DNA.[81,82] These proteins may act by displacing histones H2A-H2B[80] which are deficient in transcriptionally active chromatin.

Experiment in the chicken globin and ovalbumin systems suggest that these genes may become sensitive to digestion by DNase I before the onset of transcription and, at least over short time periods, remain DNase I sensitive after transcription ceases.[75,83] A similar observation in the vitellogenin system might help to explain the shorter lag period observed on secondary estrogen stimulation. If the vitellogenin genes in withdrawn *X. laevis* remain in an "open" transcriptionally active conformation they might be immediately accessible for initiation of transcription — resulting in a shorter lag period. The susceptibility of the vitellogenin genes to DNase I digestion has been examined following partial DNase I digestion of isolated nuclei. DNA was extracted, fractionated into four size classes, and the relative amount of vitellogenin DNA in each size class was determined by moderate cDNA excess hybridization to vitellogenin cDNA. DNA which is preferentially digested by DNase I becomes enriched in the smallest of the four size classes.[75] In unstimulated cells, which do not contain detectable vitellogenin mRNA and do not transcribe the vitellogenin genes at a significant rate, the two lower molecular weight fractions are deficient in vitellogenin DNA. Administration of estradiol-17β induces transcription of the vitellogenin genes and results in a dramatic increase in the abundance of vitellogenin sequences in the most rapidly degraded, smallest size class of DNA. Long-term estrogen withdrawal (2 months) results in complete restoration of the DNase I digestion pattern observed in unstimulated cells.[54,84] These results indicate that in unstimulated cells the vitellogenin genes are in the transcriptionally inactive bulk chromatin which is relatively insensitive to digestion by DNase I. Administration of estrogen induces transcription of the vitellogenin genes and converts the vitellogenin genes into a transcriptionally active DNase I sensitive conformation. Estrogen administration does not produce a permanent change in the conformation of the vitellogenin genes in chromatin. The vitellogenin genes in withdrawn chromatin have returned to the transcriptionally inactive DNase insensitive conformation found in unstimulated cells. The data indicate that the shorter lag period observed during secondary stimulation is not the result of permanent conversion of the vitellogenin genes to a more "open" conformation — at least with respect to those structural features which are susceptible to investigation using DNase I. The utility of the vitellogenin system, in which a reversible regulatory response occurs in a stable cell type, is also apparent as it allowed the first demonstration that conversion of a gene into a DNase I sensitive conformation is reversible and can occur in the absence of DNA replication.[54,84] At this time it is not known whether conversion of the vitellogenin genes into a more open DNase I sensitive conformation is a prerequisite for RNA polymerase binding and transcription, or is a consequence of the passage of RNA polymerase down the vitellogenin DNA.

VII. VITELLOGENIN GENE TRANSCRIPTION, mRNA PROCESSING AND STABILITY

A. Vitellogenin Gene Transcription in Heterologous Cells

Vitellogenin mRNA is absent in unstimulated *Xenopus* liver cells[45,46,48] and is not found in cultured *Xenopus* kidney cells.[55] or in oocytes.[105] The availability of cloned vitellogenin cDNAs makes possible sensitive measurements of both relative and absolute rates of gene transcription. In essence, nuclear RNA is pulse labeled in whole cells or in isolated nuclei, hybridized to DNA immobilized on nitrocellulose filters and

the hybridized material is quantitated after extensive washing and digestion.[85] This type of study is only beginning in the vitellogenin system. Brock et al.[55] have demonstrated that the *Xenopus* vitellogenin gene is not transcribed at a low rate in nonhepatic cells. Each of the four vitellogenin genes is transcribed at a rate which does not exceed 0.6 ppm (relative to total nuclear RNA synthesis) in nuclear RNA from *Xenopus* kidney cells cultured in both the presence and absence of estrogen. Since these cells do contain vitellogenin DNA, the transcription of the vitellogenin genes is not "leaky" as has been suggested for the genes coding for all nonhyperprevalent mRNAs.[86]

B. Processing of Vitellogenin mRNA Precursors

Investigations of the vitellogenin mRNA precursors are still at an early stage. Ryffel et al.[68] have described large polyadenylated precursors to vitellogenin mRNA which suggests that polyadenylation occurs very early in processing. In contrast Jost et al.[70] have described a precursor to avian vitellogenin mRNA which is only 5 to 10% polyadenylated. The data for *Xenopus* vitellogenin mRNA precursors[68] suggest that polyadenylation precedes splicing. Although a great deal of work will obviously be needed to characterize the splicing out of the 33 intervening sequences in a single vitellogenin mRNA precursor, their data led Ryffel et al. to suggest that processing out of intervening sequences does not follow a single pathway.

C. Stability of Cytoplasmic Vitellogenin mRNA

Despite its size, cytoplasmic vitellogenin mRNA appears to be relatively stable during estrogen stimulation. Vitellogenin mRNA labeled in vivo did not decay appreciably when it was isolated many hours later.[43] The accumulation of vitellogenin mRNA during primary stimulation[22] exhibits the linear kinetics characteristic of a species which is being synthesized but not degraded.[87,88] When the half-life of vitellogenin mRNA during primary induction[22] is calculated using the expression of Kafatos[87,88] vitellogenin mRNA exhibits a half-life of approximately 800 hr during estrogen stimulation and 45 hr during early withdrawal.[106]The selective stabilization of major hormonally regulated mRNAs may be widespread as hormonal stabilization of mouse casein mRNAs,[89] and hen ovalbumin mRNA[90] has been reported. The absence of substantial cell proliferation and cell death makes the vitellogenin system an attractive one in which to pursue studies of hormone induced stabilization of mRNA.

VIII. CELLULAR SYNTHESIS OF VITELLOGENIN

The induction of vitellogenin synthesis requires a major reorganization of cell structures as well as synthesis of new cell components. Vitellogenin is a secreted phospholipoglycoprotein which is synthesized on membrane bound polyribosomes. The massive induction of vitellogenin synthesis in *Xenopus* liver is characterized by proliferation of the endoplasmic reticulum and Golgi apparatus[18,91] by induction of new ribosomes and ribosomal proteins,[107] and by a doubling of total cell RNA content.[33,42] This complex of events in essence represents a specialization of liver cells for the production of and secretion of massive amounts of a specific protein. The lower level of induced vitellogenin synthesis in rooster liver results in a correspondingly smaller cellular response.

Vitellogenin synthesizing polysomes containing 30 to 40 ribosomes have been identified in both *Xenopus*[21,33,92] and rooster.[35,93]

The question of whether or not the translational activity of vitellogenin mRNA is regulated along with its level is controversial. Farmer et al.[30,94] have reported that the induction of large amounts of translatable vitellogenin mRNA procedes the appearance

of that RNA in immunoprecipitable polysomes during primary estrogen stimulation of male *Xenopus laevis*. They report a close correspondence between these parameters during secondary estrogen stimulation. In other studies in which either in vitro translation or hybridization to cDNA was employed to quantitate vitellogenin mRNA, and immunoprecipitation was employed to determine rates of vitellogenin synthesis, no substantial translation control was observed.[38,42,95] Given the difficulty in achieving quantitative antibody precipitation of polysomes[96,97] it seems fair to conclude that the major site of control in both avian and amphibian systems is at the level of the quantity of vitellogenin mRNA in cells.

IX. OTHER LIVER GENES REGULATED BY ESTROGEN

The massive induction of vitellogenin mRNA has led to investigation of estrogen effects on other liver proteins as well. In roosters apo-very low density lipoprotein (apo VLDL) is induced to almost the same level as vitellogenin.[98-100] A hybridization enrichment technique was employed to identify a cDNA clone of apo VLDL by Deeley et al.[100] In constrast to vitellogenin, apo VLDL is synthesized at an appreciable rate in the absence of estrogen.

Given the massive synthesis and secretion of vitellogenin and the accumulation of vitellogenin in the serum of male animals, considerable attention has centered on the production of the major serum protein, serum albumin, during the estrogen induction of vitellogenin. Albumin synthesis in avian liver declines by 50% following primary estrogen stimulation.[101] Farmer et al. suggested that the level of translatable albumin mRNA remains fairly constant during primary estrogen stimulation but declines during secondary estrogen stimulation.[94] In constrast, Wangh et al. who examined the proteins secreted into the medium by primary monolayers of *Xenopus* liver cells exposed to estrogen, reported the total disappearance of albumin within a few days.[40] Recently, Philipp et al.[102] examined albumin mRNA levels by measuring the hybridization of cDNA synthesized from total cell RNA to cloned albumin cDNA immobilized on nitrocellulose filters. They found that the number of molecules of albumin mRNA per cell remains constant during primary estrogen stimulation. Since both total cell RNA and cell mRNA double during primary estrogen stimulation the fraction of cell RNA which is albumin mRNA falls by approximately 50% during this period. The apparent persistence of albumin synthesis in the face of the massive induction of vitellogenin is a striking example of the ability of hepatocytes to maintain multipotential differentiated functions even in the face of the competing demands of massive vitellogenin synthesis.

X. CONCLUSIONS

Although the detailed molecular mechanisms regulating vitellogenin gene expression and mRNA levels remain obscure, several key features of these processes have been revealed in recent studies. These events are outlined for cells of male *X. laevis* in which the most detailed information is available. Unstimulated liver cells preserve the potential to respond to estrogen by transcription of the vitellogenin genes but they are in no sense primed to do so. In the liver cells of unstimulated male *Xenopus* the vitellogenin genes are insensitive to DNase and do not appear to be transcribed at an appreciable rate.[54,84] The estrogen receptors which will be required to mediate the response to estrogen are only present at minimal levels in both male *X. laevis* and roosters. When estrogen diffuses into cells it does not contact the high levels of pre-existing cytoplasmic receptors which can rapidly translocate into the nucleus that are typcial of other hormonally responsive cells.[8,9,53,57] Instead, in *Xenopus* it must diffuse into the nucleus and bind to nuclear

estrogen receptor. The estrogen-receptor complex both activates vitellogenin gene transcription and auto-induces the synthesis of the additional receptor which is required for maximal transcription of the vitellogenin genes.[56,57] While the transcription of the vitellogenin genes requires the continued presence of a hormone, the auto-induction of estrogen receptor is a permanent irreversible response of the cells to estrogen and "primes" the cells for future vitellogenin gene expression — which is both efficient and rapid.[53]

In the presence of nuclear estrogen-receptor complex the vitellogenin genes take on an open DNase I sensitive conformation either as a prerequisite for, or a consequence of, the initiation of transcription.[54,84] The large initial transcripts of the vitellogenin genes are polyadenylated[68] and the 33 intervening sequences[66-69] are removed by processing in a highly specific but nonsequential process.[68] Vitellogenin mRNA which reaches the cytoplasm interacts with the large pool of inactive ribosomes in unstimulated *Xenopus* liver cells.[21] and begins to synthesize vitellogenin which is then modified and secreted by the liver cells. Since vitellogenin mRNA appears to be extremely stable in estrogen stimulated liver cells, each mRNA molecule may be translated thousands of times. The accumulation of massive amounts of vitellogenin mRNA eventually exhausts the pool of ribosomes and ribosomal subunits and saturates the cell secretory apparatus. The induction of ribosomal RNA[45] and proteins[108] and of HMG-CoA reductase and acetyl-CoA carboxylase, the key regulatory enzymes in cholesterol and fatty acid synthesis follows.[103] Whether the level of cell components is directly regulated by estrogen-receptor complex or responds to homeostatic mechanisms which recognize their depletion is not known.

Withdrawal of estrogen evokes the reversal of most but not all of these responses. Vitellogenin mRNA is degraded and ultimately declines to undetectable levels.[45,46,48] The vitellogenin genes revert to their original "inactive" DNase I insensitive conformation.[54,84] The extensive secretory apparatus is slowly degraded and the liver cells revert to their original morphology. Estrogen receptor synthesis continues and the receptor accumulates in the cytoplasm facilitating future response to estrogen.[53]

This picture of the events involved in the estrogen induction of vitellogenin, while occasionally based on fragmentary data, seems most consistent with available information in this system. Investigations from many laboratories are providing an overview of what happens during induction of vitellogenin mRNA and it now seems possible to ask, to paraphase Bettman Hollweig, "how does it all happen?".

REFERENCES

1. **Weil, P. A., Luse, D. S., Segall, J., and Roeder, R. G.,** Selective and accurate initiation of transcription at the Ad2 major late promoter in a soluble system dependent on purified RNA polymerase II and DNA, *Cell*, 18, 469, 1979.
2. **Pelham, R. B. and Brown, D. D.,** A specific transcription factor that can bind either the 5S RNA gene or 5S RNA, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 4170, 1980.
3. **Roeder, R. G., Ng, S., Shastry, B. S., and Engelke, D. R.,** Specific interaction of a purified transcripton factor with an internal control region of 5S RNA genes, *Cell*, 19, 717, 1980.
4. **Manley, J. L., Fire, A., Cano, A., Sharp, P. A., and Gefter, M. L.,** DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 3855, 1980.
5. **Kohler, G. and Milstein, C.,** Continuous cultures of fused cells secreting antibody of predefined specificity, *Nature*, 256, 495, 1980.
6. **Kennett, R. H., Denis, K. A., Tung, A. S., and Klinman, N. R.,** Hybrid plasmacytoma production: fusions with adult spleen cells, monoclonal spleen fragments, neonatal spleen cells and human spleen cells, *Curr. Top. Microbiol. Immunol.*, 81, 77, 1978.

7. **Lubin, R. and Mohler, M.,** In vitro immunization as an adjunct to the production of hybridomas against lymphokine osteoclast activating factor, *Mol. Immunol.*, 1980, in press.
8. **Yamamoto, K. P. and Alberts, B. M.,** Steroid receptors: elements for modulation of eucaryotic transcription, *Annu. Rev. Biochem.*, 45, 722, 1976.
9. **Palmiter, R. D., Moore, P. B., Mulvihill, E. R., and Emtage, S.,** A significant lag in the induction of ovalbumin messenger RNA by steroid hormones: a receptor translocation hypothesis, *Cell*, 8: 557, 1976.
10. **Wyatt, G. R. and Pan, M. L.,** Insect plasma proteins, *Annu. Rev. Biochem.*, 47, 779, 1978.
11. **Engelman, F.,** Insect vitellogenin: identification, biosynthesis, and role in vitellogenesis, *Adv. Insect Physiol.* 14, 49, 1979.
12. **Wallace, R. A.,** The role of protein uptake in vertebrate oocyte growth and yolk formation, *Oogenesis*, Biggers, J. D. and A. W. Schultz, Eds., University Park Press, Baltimore, 1972, 339.
13. **Wallace, R. A. and Jared, D. W.,** Studies on amphibian yolk. VII. Serum phosphoprotein synthesis by vitellogenic females and estrogen-treated males of *Xenopus laevis*, *Can. J. Biochem.*, 46, 953, 1968.
14. **Wallace, R. A. and Jared, Donald W.,** Studies on amphibian yolk VIII. The estrogen-induced hepatic synthesis of a serum lipophosphoprotein and its selective uptake by the ovary and transformation into yolk platelet proteins in *Xenopus laevis*, *Dev. Biol.*, 19, 498, 1969.
15. **Bergink, E. W. and Wallace, R. A.,** Precursor-product relationship between amphibian vitellogenin and the yolk proteins, lipovitellin and phosvitin, *J. Biol. Chem.*, 249, 2897, 1974.
16. **Dolphin, P. J., Ansari, A. Q., Lazier, C. B., Munday, K. A., and Akhtar, M.,** Studies on the induction and biosynthesis of vitellogenin, an oestrogen-induced glycoliphosphoprotein, *Biochem. J.*, 124, 751, 1971.
17. **Wittliff, J. L. and Kenney, F. T.,** Regulation of yolk protein synthesis in amphibian liver I. Induction of lipovitellin synthesis by estrogen, *Biochim. Biophys. Acta.*, 269, 485, 1972.
18. **Follett, B. K., Nicolls, T. J., and Redshaw, M. R.,** The vitellogenic response in the South African clawed toad (*Xenopus laevis* Daudin), *Cell Physiol.*, 72, 91, 1968.
19. **Deeley, R. G., Mullinix, K. P., Wetekam, W., Kronenberg, H. M., Meyers, M., Elridge, J. D., and Goldberger, R. F.,** Vitellogenin is the precursor of the egg yolk phosphoproteins, *J. Biol. Chem.*, 250, 9060, 1975.
20. **Gordon, J. I., Deeley, R. G., Burns, A. T. H., Paterson, B. M., Christmann, J. L., and Goldberger, R. F.,** In vitro translation of avian vitellogenin messenger RNA, *J. Biol. Chem.*, 252, 8320, 1977.
21. **Berridge, M. V., Farmer, S. R., Green, C. D., Henshaw, E. D., and Tata, J. R.,** Characterization of polysomes from *Xenopus* liver synthesizing vitellogenin and translation of vitellogenin and albumin messenger RNAs in vitro, *Eur. J. Biochem.*, 62, 161, 1976.
22. **Shapiro, D. J., Baker, H. J., and Stitt, D. T.,** In vitro translation and estradiol-17β induction of *Xenopus laevis* vitellogenin messenger RNA, *J. Biol. Chem.*, 251, 3105, 1976.
23. **Deeley, R. G. and Goldberger, R. F.,** Regulation of expression of the vitellogenin gene in avian liver, in *Ontogeny of Receptors and Reproductive Hormone Action*, Hamilton, T. H., Clark, J. H. and Sadler, W. A., Eds., Raven Press, New York, 1979, 291.
24. **Wiley, W. H. and Wallace, R. A.,** Three different molecular weight forms of the vitellogenin peptide from *Xenopus laevis*, *Biochem. Biophys. Res. Commun.*, 85, 153, 1978.
25. **Wiley, W. H.,** Ph.D. thesis, University of Tennessee, 1979.
26. **Williams, D. L., Wang, S. Y., and Capony, F.,** Multiple response patterns to estrogenic stimulation in the avian liver, *J. Steroid Biochem.*, 11, 231, 1979.
27. **Follett, B. K. and Redshaw, M. R.,** The physiology of vitellogenesis, in *Physiology of Amphibia*, Lofts, B., Ed., Academic Press, New York, 219, 1974.
28. **Clemens, M. J.,** The regulation of egg-yolk protein synthesis by steroid hormones, *Prog. Biophys. Mol. Biol.*, 28, 71, 1974.
29. **Bergink, E. W. and Wallace, R. A.,** Estrogen-induced synthesis of yolk protein in rooster, *Am. Zool.*, 14, 1177, 1974.
30. **Tata, J. R.,** The expression of the vitellogenin gene, *Cell*, 9, 1, 1976.
31. **Tata, J. R.,** Regulation of vitellogenesis by estrogen, in *Hormones and Cell Regulation*, Dumont, J. and Nunez, J., Eds., Elsevier/North-Holland, Amsterdam, 37, 1978.
32. **Gruber, M., Bos, E. S., and Ab, G.,** Hormonal control of vitellogenin synthesis in avian liver, *Mol. Cell. Endocrinol.*, 5, 41, 1976.
33. **Skipper, J. K. and Hamilton, T. H.,** Regulation by estrogen of the vitellogenin gene, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 2384, 1977.
34. **Deeley, R. G., Gordon, J. I., Burns, A. T. H., Mullinix, K. P., Bina-Stein, M., and Goldberger, R. F.,** Primary activation of the vitellogenin gene in the rooster, *J. Biol. Chem.*, 252, 8310, 1977.
35. **Jost, J.-P., Ohno, T., Panyim, S., and Schuerch, A. R.,** Appearance of vitellogenin mRNA sequences in chicken liver following primary and secondary stimulation by 17β-estradiol, *Eur. J. Biochem.*, 84, 355, 1978.

36. **Green, C. D. and Tata, J. R.,** Direct induction by estradiol of vitellogenin synthesis in organ cultures of male *Xenopus laevis, Cell,* 7, 131, 1976.
37. **Wangh, Lawrence, J. and Knowland, John,** Synthesis of vitellogenin in cultures of male and female frog liver regulated by estradiol treatment in vitro, *Proc. Nat. Acad. Sci. U.S.A.,* 72, 3172, 1975.
38. **Shapiro, D. J. and Baker, H. J.,** Estrogen regulation of *Xenopus laevis* vitellogenin gene expression, in *Ontogeny of Receptors and Reproductive Hormone Action,* Hammilton, T. H., Clark, J. H., and Sadler, W. A., Eds., Raven Press, New York, 1979, 309.
39. **Stanchfield, J. E. and Yager, J. D., Jr.,** An estrogen responsive primary amphibian liver cell culture system, *Exp. Cell Res.,* 116, 239, 1978.
40. **Wangh, L. J., Osborne, J. A., Hentschel, C. L., and Tilly, R.,** Parenchymal cells purified from *Xenopus* liver and maintained in primary culture synthesize vitellogenin in response to 17β-estradiol and serum albumin in response to dexamethasone, *Dev. Biol.,* 70, 479, 1979.
41. **Shapiro, D. J.,** Estrogen specificity in the induction of vitellogenin mRNA, manuscript in preparation.
42. **Shapiro, D. J. and Baker, H. J.,** Purification and characterization of *Xenopus laevis* vitellogenin messenger RNA, *J. Biol. Chem.,* 252, 5244, 1977.
43. **Wahli, W., Wyler, T., Weber, R., and Ryffel, G. U.,** Size, complexity and abundance of a specific poly(A)-containing RNA of liver from male *Xenopus* induced to vitellogenin synthesis by estrogen, *Eur. J. Biochem.,* 66, 457, 1976.
44. **Jost, J.-P., Pehling, G., Panyim, S., Ohno, T., and Seldran, M.,** An improved method for isolation of active vitellogenin messenger RNA from chicken liver. Use of diethylpyrocarbonate, *Biochem. Biophys. Acta,* 517, 388, 1978.
45. **Baker, H. J. and Shapiro, D. J.,** Kinetics of estrogen induction of *Xenopus laevis* messenger RNA as measured by hybridization to complementary DNA, *J. Biol. Chem.,* 252, 8428, 1977.
46. **Ryffel, G. U., Wahli, W., and Weber, R.,** Quantitation of vitellogenin messenger RNA in the liver of male *Xenopus* toads during primary and secondary stimulation by estrogen, *Cell,* 11, 213, 1977.
47. **Deeley, R. G., Udell, D. S., Burns, A. T. H., Gordon, J. I., and Goldberger, R. F.,** Kinetics of avian vitellogenin messenger RNA induction, *J. Biol. Chem.,* 252, 7913, 1977.
48. **Baker, H. J. and Shapiro, D. J.,** Rapid accumulation of vitellogenin messenger RNA during secondary estrogen stimulation of *Xenopus laevis, J. Biol. Chem.,* 253, 4521, 1978.
49. **Burns, A. T. H., Deeley, R. G., Gordon, J. I., Udell, D. S., Mullinix, K. P., and Goldberger, R. F.,** Primary induction of vitellogenin mRNA in the rooster by 17β-estradiol, *Proc. Nat. Acad. Sci. U.S.A.,* 75, 1815, 1978.
50. **Jensen, E. V. and DeSombre, E. R.,** Estrogen-receptor interaction, *Science,* 182, 126, 1973.
51. **Katzenellenbogen, B. S. and Gorski, J.,** Estrogen actions on synthesis in target cells, in *Biochemical Actions of Hormones,* Litwak, G., Ed., Academic Press, New York, 188, 1975.
52. **Westley, B. and Knowland, J.,** An estrogen receptor from *Xenopus laevis* liver possibly connected with vitellogenin synthesis, *Cell,* 15, 367, 1978.
53. **Hayward, M. A., Mitchell, R. A., and Shapiro, D. J.,** Induction of estrogen receptor and reversal of the nuclear-cytoplasmic receptor ratio during vitellogenin synthesis and withdrawal in *Xenopus laevis, J. Biol. Chem.,* 255, 11308, 1980.
54. **Folger, K. and Shapiro, D. J.,** The vitellogenin genes in *Xenopus laevis* acquire and lose DNase I sensitivity during estrogen stimulation and withdrawal, 1980, manuscript in preparation.
55. **Brock, M., Hayward, M. A., Folger, K., and Shapiro, D. J.,** The vitellogenin genes are not transcribed at a significant rate in cultured *Xenopus laevis* kidney cells, 1980, submitted for publication.
56. **Westley, B. and Knowland, J.,** Estrogen causes a rapid, large and prolonged rise in the level of nuclear estrogen receptor in *Xenopus laevis* liver, *Biochem. Biophys. Res. Commun.,* 88, 1167, 1979.
57. **Lazier, C. B. and Haggarty, A. J.,** A high-affinity estrogen-binding protein in cockerel liver cytosol, *Biochem. J.,* 180, 347, 1979.
58. **Kastern, W., Eldridge, J. D., and Mullinix, K. P.,** DNase I digestion of chromatin from avian liver nuclei liberates DNA-dependent RNA polymerase II, *J. Biol. Chem.,* 254, 7368, 1979.
59. **Gschwendt, M. and Schneider, W.,** Estrogen-binding sites of chicken liver, *Eur. J. Biochem.,* 91, 139, 1978.
60. **Gschwendt, M.,** Solubilization of the chromatin-bound estrogen receptor from chicken liver and fractionation on hydroxylapatite, *Eur. J. Biochem.,* 67, 411, 1976.
61. **Witliffe, J. L. and Zelson, P. R.,** Estrogen specificity of the induction of lipovitellin synthesis and evidence for a specific estrogen binding component, *Endocrinol. Res. Commun.,* 1, 117, 1974.
62. **Kowalczykowski, S. C., Lonberg, N., Newport, J., and Von Hippel, P.,** On the nature and specificity of the interaction of T4-coded gene 32-protein with nucleic acids, *Biophysical Discussions of the Biophysical Society,* 1980, in press.
63. **Ryffel, G. U.,** Comparison of cytoplasmic and nuclear poly(A)-containing RNA sequences in *Xenopus* liver cells, *Eur. J. Biochem.,* 62, 417, 1976.

64. **Wahli, W., Ryffel, G. U., Wyler, T., Jaggi, R. B., Weber, R., and Dawid, I. B.,** Cloning and characterizaton of synthetic sequences from the *Xenopus laevis* vitellogenin structural gene, *Dev. Biol.*, 67, 371, 1978.
65. **Wahli, W., Dawid, I. B., Wyler, T., Jaggi, R. B., Weber, R., and Ryffel, G. U.,** Vitellogenin in *Xenopus laevis* is encoded in a small family of genes, *Cell*, 16, 535, 1979.
66. **Wahli, W. and Dawid, I. B.,** Isolation of two closely related vitellogenin genes, including their flanking regions, from a *Xenopus laevis* gene library, *Proc. Natl. Acad. Sci.*, 77, 1437, 1980.
67. **Wahli, W., Dawid, I. B., Wyler, T., Weber, R., and Ryffel, G. U.,** Comparative analysis of the structural organization of two closely related vitellogenin genes in *Xenopus laevis*, *Cell*, 20, 107, 1980.
68. **Ryffel, G. U., Wyler, T., Muellener, D. B., and Weber, R.,** Identification organization and processing intermediates of the putative precursors of *Xenopus* vitellogenin messenger RNA, *Cell*, 19, 53, 1980.
69. **Wahli, W., Dawid, I. B., Ryffel, G. U., and Weber, R.,** Vitellogenesis and vitellogenin gene family, *Science*. In press.
70. **Jost, J.-P., Pehling, G., Ohno, T., and Cozens, P.,** Identification of a large precursor of vitellogenin mRNA in the liver of estradiol-treated chicks, *Nucleic Acids Res.*, 5, 4781-4793, 1978.
71. **Ohno, T., Cozens, P. J., Cato, A. C. B., and Jost, J.-P.,** Recombinant plasmids containing avian vitellogenin structural gene sequences derived from complementary DNA. *Biochim. Biophys. Acta*, 606, 34, 1980.
72. **Kastern, W. H., Christmann, J. L., Eldridge, J. D., and Mullinix, K. P.,** Estrogen regulates the number of RNA polymerase II molecules in rooster liver, *Biochemistry*, 1980, submitted for publication.
73. **Dierks-Ventling, C.,** In vitro transcription of vitellogenin sequences on chick liver chromatin, *Nucleic Acids Res.*, 5, 3929, 1978.
74. **Alberga, A., Tran, A., and Baulieu, E. E.,** Distribution of estradiol receptor and vitellogenin gene in chick liver chromatin fractions, *Nucleic Acids Res.*, 7, 2031, 1979.
75. **Weintraub, H. and Groudine, M.,** Chromosomal subunits in active genes have an altered conformation, *Science*, 193, 848, 1976.
76. **Lacy, E. and Axel, R.,** Analysis of DNA of isolated chromatin subunits, *Proc. Natl. Acad. Sci.*, 72, 3978, 1975.
77. **Garel, A. and Axel, R.,** Selective digestion of transcriptionally active ovalbumin genes from oviduct nuclei, *Proc. Natl. Acad. Sci.*, 73, 3966, 1976.
78. **Bloom, K. S. and Anderson, J. N.,** Fractionation of hen oviduct chromatin into transcriptionally active and inactive regions after selective micrococcal disease digestion, *Cell*, 15, 141, 1978.
79. **Bonner, J., Gottesfeld, J., Garrard, W., Billing, R., and Uphouse, L.,** Isolation of template active and inactive regions of chromatin, *Methods Enzymol.*, 40B, 97, 1975.
80. **Weisbrod, S., Groudine, M., and Weintraub, H.,** Interaction of HMG 14 and 17 with actively transcribed genes, *Cells*, 19, 289, 1980.
81. **McGhee, J. D. and Ginder, G. D.,** Specific DNA methylation sites in the vicinity of the chicken -globin genes, *Nature*, 280, 418, 1979.
82. **Razin, A. and Riggs, A. D.,** DNA methylation and gene function, *Science*, 210, 604, 1980.
83. **Palmiter, R. D., Mulvihill, E. R., McKnight, G. S., and Senear, A. W.,** Regulation of gene expression in the chick oviduct by steroid hormones, *Cold Spring Harbor Symp. Quant. Biol.*, 42, 639, 1977.
84. **Folger, K. R., Anderson, J. N., and Shapiro, D. J.,** Nuclease sensitivity of the vitellogenin genes in stimulated and unstimulated *Xenopus* liver, *Fed. Proc.*, 39, 1886, 1980.
85. **McNight, S. G. and Palmiter, R. D.,** Transcriptional regulation of the ovalbumin and conalbumin genes by steroid hormones in chick oviduct, *J. Biol. Chem.*, 254, 9050, 1979.
86. **Davidson, E. H. and Britten, R.,** Regulation of gene expression; possible role of repetitive sequences, *Science*, 204, 1052, 1979.
87. **Kafatos, F.,** mRNA stability and cellular differentiation, Acta Endocrinol. Suppl., 168, 319, 1972.
88. **Schimke, R. T. and Doyle, D.,** Control of enzyme levels in animal tissue, *Ann. Rev. Biochem.*, 39, 929, 1970.
89. **Rosen, J. M., Gruyette, W. A., and Matusik, R. J.,** Prolactin-mediated transcriptional and post-transcriptional control of casein gene expression, *Cell*, 17, 1013, 1979.
90. **Palmiter, R. D. and Carey, N. H.,** Rapid inactivation of ovalbumin messenger ribonucleic acid after acute withdrawal of estrogen, *Proc. Natl. Acad. U.S.A.*, 71, 2357, 1974.
91. **Lewis, J. A., Clemens, M. J., and Tata, J. R.,** Morphological and biochemical changes in the hepatic endoplasmic reticulum and golgi apparatus of male *Xenopus laevis* after induction of egg-yolk protein synthesis by estradiol-17β, *Mol. Cell. Endocrinol.*, 4, 311, 1976.
92. **Swiderski, R. E., Johnson, S. A., Larkens, B. A., and Graham, D. E.,** Sequential sepharose chromatographic isolation of polysomes and polysomal RNAs depleted in nuclear RNA from *Xenopus, Nucleic Acids Res.*, 6, 3685, 1979.

93. **Dijkstra, J., Touw, J., Halsema, I., Gruber, M., and Ab, G.,** Estradiol-induced synthesis of vitellogenin IV. The isolation of non-degraded polysomes from avian liver using an endogenous ribonuclease inhibitor, *Biochim. Biophys. Acta,* 521, 363, 1978.
94. **Farmer, S. R., Henschaw, E. C., Berridge, M. V., and Tata, J. R.,** Translation of Xenopus vitellogenin mRNA during primary and secondary induction, *Nature,* 273, 401.
95. **Felber, B. K., Ryffel, G. U., and Weber, R.,** Estradiol-induced accumulation of vitellogenin mRNA and secretion of vitellogenin in liver cultures of *Xenopus, Mol. and Cell. Endocrinol.,* 12, 151, 1978.
96. **Shapiro, D. J., Taylor, J. M., McNight, S. G., Palacios, R., Gonzales, C., Kiely, M. L., and Schimke, R. T.,** Isolation of hen oviduct ovalbumin and rat liver albumin polysomes by indirect immunoprecipitation, *J. Biol. Chem.,* 249, 3665, 1974.
97. **Shapiro, D. J. and Schimke, R. T.,** Immunochemical isolation and characterization of ovalbumin messenger RNA, *J. Biol. Chem.,* 250, 1759, 1975.
98. **Chan, L., Snow, L. D., Jackson, R. L., and Means, A. R.,** Hormonal regulation of lipoprotein synthesis in the cockerel, in *Ontogeny of Receptors and Reproductive Hormone Action,* Hamilton, T. H., Clark, J. H., and Sadler, W. A. Eds., Raven Press, New York, 1979, 331.
99. **Wiskocil, R., Bensky, P., Dower, W., Goldberger, R. F., Gordon, J. I., and Deeley, R. G.,** Coordinate regulation of two estrogen-dependent genes in avian liver. *Proc. Natl. Acad. Sci. U.S.A.,* 77, 4474, 1980.
100. **King, C. R., Udell, D. S., and Deeley, R. G.,** Characterization of the estrogen-responsive domain of avian liver and cloning of double-stranded cDNA derived from estrogen-inducible RNA species, *J. Biol. Chem.,* 254, 6781, 1979.
101. **Williams, D. L., Wang, S-Y., and Klett, H.,** Decrease in functional albumin mRNA during estrogen induced vitellogenin biosynthesis in avian liver, *Proc. Natl. Acad. Sci. U.S.A.,* 75, 5974, 1978.
102. **Philipp, B. P., Morgan, E. A., and Shapiro, D. J.,** Cloning of *Xenopus laevis* albumin cDNA and measurement of estrogen effects on albumin mRNA levels, submitted for publication, 1980.
103. **Phillip, B. P. and Shapiro, D. J.,** Estrogen induction of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase and acetyl CoA carboxylase in *Xenopus laevis, J. Biol. Chem.,* 256, 2922, 1981.
104. **Hayward, M. and Shapiro, D. J.,** unpublished observations.
105. **Rosbash, M.,** personal communication.
106. **Brock, M. and Shapiro, D.,** unpublished.
107. **Rosbash, M.,** personal communication.
108. **Rosbash, M.,** personal communication.