Transferrin Gene Expression

REGULATION OF mRNA TRANSCRIPTION IN CHICK LIVER BY STEROID HORMONES AND IRON DEFICIENCY*

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G. Stanley McKnight,‡ David C. Lee,§ and Richard D. Palmiter
From the Howard Hughes Medical Institute Laboratory, Department of Biochemistry, University of Washington, Seattle, Washington 98195

Estrogen and iron deficiency regulate the synthesis of transferrin in chick liver by causing a specific increase in transcription of the transferrin gene. The relative rate of synthesis of transferrin mRNA was measured by allowing isolated nuclei to continue transcription in vitro in the presence of [α-32P]UTP. The 32P transcripts then were hybridized to immobilized recombinant DNA containing the double-stranded transferrin cDNA sequence. Transcripts from control liver nuclei contained 0.009% transferrin mRNA; iron deficiency and estrogen treatment caused an increase in the rate of transferrin mRNA synthesis of 1.5- and 2-fold, respectively. These increases in the transcriptional activity of the transferrin gene were accompanied by increases in the cellular level of transferrin mRNA, the rate of transferrin synthesis, and the concentration of serum transferrin. The combined treatment of iron deficiency and estrogen resulted in a synergistic response in all parameters of transferrin induction, including the rate of transferrin mRNA synthesis which increased 3.2-fold to 0.029% of total RNA synthesis. Although estrogen stimulation leads to a 40% decrease in liver non-heme iron, the estrogen-mediated induction of transferrin mRNA was not blocked when liver iron was maintained at high levels with injected ferritin. These results suggest that iron deficiency and estrogen interact with the liver transferrin gene through separate regulatory mechanisms. The transferrin gene is also expressed in oviduct and we compare and discuss the tissue-specific response of this gene to iron and steroid hormones.

Chick liver synthesizes and secretes transferrin which functions as the principal iron transport protein of serum; hormone-stimulated chick oviduct also produces a transferrin (frequently referred to as conalbumin) which is secreted and becomes a major egg white protein. In 1962, Williams (1) suggested that these two proteins are products of the same gene based on immunological cross-reactivity, peptide mapping, and electrophoretic variants. The identity of liver and oviduct transferrin has been substantiated more recently by comparative amino acid sequencing of their NH2-terminal signal peptides (2) and cDNA cross-hybridization studies (3).

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† Present address, Dept. of Pharmacology, University of Washington, Seattle, Wash. 98195.
§ Present address, Dept. of Biological Chemistry, Washington University, St. Louis, Mo. 63110.

In addition, a recombinant plasmid has been constructed which contains the transferrin cDNA sequence (4) and this has allowed us to analyze the structural organization of the transferrin gene in liver and oviduct DNA and to perform a direct determination of the gene copy number. The results confirmed the presence of a single copy of the gene per haploid genome and we could find no detectable differences in the structural organization of the gene in liver and oviduct DNA when analyzed by restriction endonuclease mapping (5).

Despite the fact that liver and oviduct transferrin mRNAs are transcribed from the same gene, there are important differences in the regulation of transferrin expression in the two tissues. As described in the preceding paper, nutritional iron deficiency leads to an increase in transferrin synthesis and transferrin mRNA in liver but has no effect on the levels of oviduct transferrin mRNA (6). Both tissues respond to steroid hormones, although the magnitude and kinetics of induction differ considerably. The induction of oviduct transferrin mRNA by estrogen begins within 1 h and the level of transferrin mRNA increases more than 50-fold after 4 days of estrogen treatment (3), whereas the liver responds more gradually with a 2-fold increase in transferrin mRNA after 4 days. Glucocorticoids also elicit an induction in both tissues but, as with estrogen, the response in liver is approximately 2-fold and occurs over 4 days, whereas the oviduct responds rapidly and transferrin mRNA levels increase more than 10-fold. In addition to these differences, the constitutive level of transferrin mRNA in liver is 10 to 20-fold greater than that in oviduct in the absence of steroids.

The regulation of transferrin mRNA sequences by hormones and iron levels could be mediated by changes in either the synthesis or stability of transferrin mRNA. In order to distinguish between these possibilities, hybridization techniques have been developed to measure the rates of transcription of specific genes using pulse-labeled RNA and immobilized recombinant DNA (7-9). Using this method, we have recently demonstrated that steroid hormones induce oviduct transferrin mRNA by directly increasing the rate of transcription from the transferrin gene (7). The present study extends the analysis of transferrin regulation in the liver to direct measurements of transcription rates and explores the interactions of iron deficiency and steroid hormones on this gene.

EXPERIMENTAL PROCEDURES

Some of the methods used in this study are described in the preceding paper (6); additional experimental procedures are described below.

Preparation of Transferrin cDNA and cRNA—cDNA was synthesized from chick liver nuclei using oligo(dT) as the primer. The DNA was purified by ethanol precipitation and was dissolved in 20 mM Tris (pH 7.5) and 10 mM EDTA. The cDNA was used as template for the synthesis of cRNA using T7 RNA polymerase.

1 L. Hager, G. S. McKnight, and R. D. Palmiter, manuscript in preparation.

2 The abbreviations used are: cDNA, transferrin cDNA; SDS, sodium dodecyl sulfate.
thesized from purified oviduct transferrin mRNA as previously described (3). In order to measure the efficiency of hybridization of transferrin mRNA sequences to immobilized pBR322-transferrin DNA, we prepared a 3H-labeled copy of transferrin cDNA using Escherichia coli DNA dependent RNA polymerase and [3H]UTP as previously described (7). We used this copy RNA as an internal standard in all filter hybridization reactions and refer to it as cRNA.

Isolation of Nuclei—Chicks were killed and the livers were removed immediately and chilled on ice. Nuclei were prepared from 0.5 to 1 g of liver as previously described (10), suspended in 40% glycerol, 50 mM Tris-CI (pH 8.3), 5 mM MgCl₂, 0.1 mM EDTA at 1 mg/ml DNA, and stored at -20°C. Rapid preparation of nuclei at 0°C was essential for obtaining the most active nuclei in subsequent transcription experiments.

Transcription with Isolated Nuclei—Nuclei (about 80 μg of DNA equivalent) were incubated for 1 h at 26°C in a 100-μl reaction containing: 20 mM Tris-CI (pH 8); 25% glycerol; 5 mM MgCl₂; 0.1 mM EDTA; 0.4 mM concentrations of ATP, GTP, and CTP; and 100 pCi of [3H]cRNA, and dissolved in 0.1% SDS for use in filter hybridizations.

DNA Binding and Hybridization Conditions—A solution of plasmid DNA at 50 μg/ml in 2 m NaCl, 0.2 m NH₄OH, was boiled for 1 min to denature and introduce breaks in the circular DNA molecules. The DNA solution (4 μl) then was applied to filter discs (0.7 cm in diameter) of Sartorius nitrocellulose paper (Beckman) and the filters were washed in 20 mM Tris-CI, 2 mM EDTA, 10 mM Tris-Cl, pH 7.5 containing 1% SDS at 45°C for 30 min, and air-dried. Hybridizations were performed in 40 μl containing 50 mM 1,4-piperazinediethanesulfonic acid (pH 7.8), 0.5 mM NaCl, 2 mM EDTA; 0.4% SDS; 52% formamide, 500 cpm of [3H]cRNA, and three filters containing wild type pBR322, pBR322-transferrin, and pCR1-ovalbumin DNA. The reactions were overlaid with paraffin oil and incubated at 45°C for 2 days. Following the hybridization, filters were washed for 2 h at 45°C with Buffer A containing 0.1% SDS, digested with RNase A (10 μg/ml) and RNase T1 (1 μg/ml) in Buffer A at 37°C for 30 min, and washed again at 45°C as before. The filters then were incubated for 15 min with 250 μl of 0.04 M NaOH and neutralized with 100 μl of 0.1 M acetic acid, and the radioactivity was determined after the addition of 4 ml of scintillation fluid (25% Triton X-100, 75% xylene, 4 g/liter of Omnifluor (New England Nuclear)).

Recombinant DNA—The recombinant plasmid containing ovalbumin cDNA sequences (pCR1-ovalbumin) was isolated by Humphries et al. (11) and contains all but 75 nucleotides at the 5' end of the mRNA sequence (12). The recombinant plasmid, pBR322-transferrin (originally referred to as pBR322-con 1), was obtained in collaboration with the laboratory of P. Chambon (Faculté de Médecine, Strasbourg, France) and contains a 2360-nucleotide segment of transferrin (conalbumin) DNA with only 20 nucleotides at the 5' end of the mRNA sequence missing (4). These recombinant plasmids were grown under P2-EK1 containment as specified by The National Institutes of Health guidelines and approved by the University of Washington Institutional Bioshazards Committee. Plasmid DNA was prepared as described (4).

RESULTS

Regulation of the Transferrin Gene is Tissue-specific—Previous work with rats suggested that, in addition to liver, many highly differentiated tissues (spleen, bone marrow, kidney, lung, and brain) might be capable of synthesizing transferrin (13, 14). We have examined that possibility in chickens using cDNA hybridization to detect transferrin mRNA. This method is both sensitive and specific, allowing the detection of as few as 5 molecules of transferrin mRNA/cell. The results shown in Table I demonstrate that only liver and estrogen-stimulated oviduct contain large amounts of transferrin mRNA (700 and 4900 molecules/cell, respectively). Hormone-withdrawn oviduct contains 65 molecules of transferrin mRNA/cell and transferrin synthesis is detectable at about 1% of total protein synthesis in this tissue (3). The presence of transferrin mRNA sequences in brain (45 molecules/cell) is surprising and we do not know whether this mRNA is functional in protein synthesis. Perhaps the transferrin found in cerebrospinal fluid (16) is synthesized in brain and is necessary for the transport of iron across the blood/brain barrier. The other tissues examined including kidney, pancreas, spleen, small intestine, heart, and lung contain very low or undetectable levels of transferrin mRNA sequences.

We then compared the effects of estrogen and iron deficiency on transferrin gene expression in the two principal sites of synthesis, liver and oviduct. Groups of 12 chicks were given either no hormone treatment; 10 days of estrogen followed by 10 days of hormone withdrawal; or 10 days of estrogen, 6 days of withdrawal, and 4 days of estrogen restimulation. In addition, half of the chicks in each group were raised on low iron diet and half on iron-supplemented diet. None of the treatments caused significant effects on the growth of the birds (Table II). As previously documented, the oviduct responded to estrogen with a dramatic increase in wet weight, RNA content (18), and transferrin mRNA (3); there were no detectable effects of iron deficiency on any of these parameters in oviduct (Table II).

The response of the liver to estrogen and iron deficiency, however, is more complex. Liver wet weight and the RNA/DNA ratio increased with estrogen but did not change with iron deficiency. Serum transferrin increased slightly (~1.4-fold) with estrogen but showed a much larger increase (~4-fold) in response to iron deficiency. The level of transferrin mRNA increased from 940 molecules/liver cell to 2300 and 2010 molecules/cell with estrogen and iron deficiency, respectively. When estrogen was given to iron-deficient chicks, the response appeared to be synergistic, with transferrin mRNA reaching 4000 molecules/cell as shown in Table II.

Also included in Table II are values for estrogen-withdrawn oviduct and liver. After 10 days of withdrawal, the estrogen-mediated effects on oviduct and liver have declined significantly and, in most cases, approach the values for unstimulated tissue. However, the level of transferrin mRNA in withdrawn liver remains about 1.5-fold higher than the unstimulated level. This may indicate that 10 days of withdrawal was not sufficient to completely eliminate the estrogen-induced response; alternatively, exposure to estrogen may have reversibly altered the proportion of liver cells which synthesize...
The combined effects of estrogen and iron deficiency on transferrin regulation

Groups of 4-day-old chicks were given no estrogen treatment (no hormone), 10 days of diethylstilbestrol pellets followed by 10 days of withdrawal (estrogen-withdrawn), or 10 days of diethylstilbestrol pellets, 6 days of withdrawal, and 4 days of restimulation with diethylstilbestrol pellets (estrogen-stimulated). In addition, half of the birds in each group were raised on low iron diet (--iron) and half on iron-supplemented diet (+iron). Serum transferrin and transferrin mRNA levels were determined as described (6). The RNA/DNA ratio was calculated from the concentration of total nucleic acid (determined spectrophotometrically) and the concentration of DNA (15). Values shown are the average of six birds.

<table>
<thead>
<tr>
<th>Hormone treatment</th>
<th>Diet</th>
<th>Body weight</th>
<th>Serum transferrin</th>
<th>Serum transferrin mRNA</th>
<th>Transferrin mRNA</th>
<th>RNA/DNA ratio</th>
<th>Wet weight</th>
<th>RNA/DNA ratio</th>
<th>Transferrin mRNA</th>
<th>molecules/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>No hormone</td>
<td>+Iron</td>
<td>85</td>
<td>2.4</td>
<td>0.7</td>
<td>4.2</td>
<td>840</td>
<td>7.5</td>
<td>0.82</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Iron</td>
<td>90</td>
<td>2.8</td>
<td>2.8</td>
<td>3.7</td>
<td>2010</td>
<td>7.5</td>
<td>1.0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Estrogen-withdrawn</td>
<td>+Iron</td>
<td>79</td>
<td>2.5</td>
<td>1.0</td>
<td>4.4</td>
<td>1370</td>
<td>21</td>
<td>0.54</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Iron</td>
<td>91</td>
<td>2.9</td>
<td>2.8</td>
<td>4.9</td>
<td>3030</td>
<td>34</td>
<td>0.88</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Estrogen-stimulated</td>
<td>+Iron</td>
<td>87</td>
<td>4.5</td>
<td>1.0</td>
<td>6.2</td>
<td>2320</td>
<td>403</td>
<td>3.5</td>
<td>3270</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-Iron</td>
<td>88</td>
<td>4.1</td>
<td>3.5</td>
<td>7.2</td>
<td>4000</td>
<td>404</td>
<td>3.8</td>
<td>3880</td>
<td></td>
</tr>
</tbody>
</table>

The combined effects of estrogen and iron deficiency on transferrin regulation

Chicks were stimulated with diethylstilbestrol pellets for 2 weeks and withdrawn for 2 weeks. Groups of three birds were restimulated for 4 days with estradiol benzoate (2 mg/day) or dexamethasone (5 mg/day) in addition to daily injections of 0.5 ml of horse spleen ferritin (38 mg/ml in 0.16 M NaCl) where indicated. Liver non-heme iron and transferrin mRNA levels were measured as described (6). The birds weighed about 350 g at the end of the experiment and the values shown are the average of three birds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver wet weight</th>
<th>Liver iron</th>
<th>Transferrin mRNA increase over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.6</td>
<td>122</td>
<td>828</td>
</tr>
<tr>
<td>Estrogen</td>
<td>19.6</td>
<td>75</td>
<td>2164</td>
</tr>
<tr>
<td>Estrogen plus ferrin</td>
<td>16.8</td>
<td>426</td>
<td>2085</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>17.4</td>
<td>73</td>
<td>1592</td>
</tr>
<tr>
<td>Dexamethasone plus ferrin</td>
<td>12.4</td>
<td>646</td>
<td>1942</td>
</tr>
<tr>
<td>Ferritin alone</td>
<td>9.9</td>
<td>743</td>
<td>865</td>
</tr>
</tbody>
</table>

17% iron by weight, is absorbed directly by the liver (21), and will reverse the iron-deficient response of the transferrin gene within 3 days (6). The ferritin was administered intravenously every 24 h during the 4-day treatment with hormones in an attempt to block the induction of transferrin mRNA. The results shown in Table III indicate that the dose of ferritin given (19 mg/bird) was sufficient to increase liver iron about 6-fold and that ferritin itself caused no change in liver wet weight or the level of transferrin mRNA. Table III also demonstrates that, when given alone, both estrogen and dexamethasone (a potent synthetic glucocorticoid) caused an increase in liver wet weight, an induction of transferrin mRNA, and a 40% decrease in liver non-heme iron. When either of these steroid hormones was administered in combination with ferritin, the induction of liver transferrin mRNA was not prevented even though the values for liver iron remained 4-fold higher than the control. We have also used iron/dextran (Imferon) in similar experiments and have observed a steroid induction of liver transferrin mRNA even though iron levels in the liver were maintained at high values.

We conclude that the steroid-mediated induction of liver

1 G. S. McKnight, D. C. Lee, and R. D. Palmiter, unpublished data.
transferrin is independent of its effects on iron metabolism. 

**Estrogen and Iron Deficiency Regulate the Transcription of the Transferrin Gene**—The increases in liver transferrin mRNA during either estrogen stimulation or iron deficiency suggested that these stimuli might directly regulate the transcription of the transferrin gene. To test this possibility, the rate of transferrin gene transcription was measured in liver nuclei isolated from birds raised on low iron or iron-supplemented diets and given the same hormone regimen as detailed in Table II. Nuclei were allowed to continue transcription in vitro in the presence of [α-32P]UTP and specific transcripts of the transferrin gene were isolated by hybridization to immobilized recombinant DNA as described under “Experimental Procedures.” Under these conditions we are essentially measuring the number of polymerases on the transferrin gene since there is little or no reinitiation. Transcription rates were determined by subtracting nonspecific radioactivity associated with filters containing wild type plasmid DNA from the radioactivity hybridized to filters containing pBR322-transferrin DNA. This value then was divided by the input radioactivity in the hybridization and expressed as a relative rate in parts per million. The efficiency of hybridization was monitored by including [3H]cRNA as an internal standard in each incubation and the rates of transcription then were corrected to 100% efficiency. In order to examine the validity of this assay, we have performed competition hybridization experiments which demonstrate that hybridization of both the 32P transcripts and the [3H]cRNA internal standard are reduced in parallel by the addition of an excess of competitor transferrin mRNA as shown in Fig. 2. In addition, the specific transcription of transferrin mRNA was completely blocked when nuclei were transcribed in the presence of 1 μg/ml of α-amanitin which selectively inhibits DNA-dependent RNA polymerase II (22) and reduces total incorporation by about 50% in liver nuclei. These experiments prove that we are measuring newly synthesized 32P transcripts which represent asymmetric transcription of the sense strand of the transferrin gene. A more detailed discussion of this method has been published recently (7).

The rates of transferrin mRNA transcription in liver nuclei are shown in Table IV. The control rate was 89 ppm or 0.0089% of total RNA synthesis after correcting for the efficiency of hybridization which was 27%. This rate increased 1.5-fold in iron-deficient liver and 2-fold in estrogen-stimulated liver, indicating that a major part of the response to these physiological inducers is at the level of increased transferrin mRNA synthesis. When iron-deficient chicks were also treated with estrogen, the increase in transferrin mRNA transcription was 3.2-fold over control; this synergistic effect of estrogen and iron deficiency on transcription rates correlates well with the increase in transferrin mRNA shown in Table II. There was no specific transcription of the ovalbumin gene in any of the liver nuclei tested, indicating that estrogen cannot activate this gene in liver as it does in oviduct.

**DISCUSSION**

Estrogen (3) and iron deficiency (6) both regulate the synthesis of transferrin in chick liver by producing a 2- to 3-fold increase in the level of transferrin mRNA. In this report we have measured the rate of transferrin mRNA synthesis directly and conclude that a major part of the hormonal and nutritional regulation of this gene in liver is at the transcriptional level. The relative rate of transferrin mRNA synthesis

![Fig. 2](http://www.jbc.org/)

**Fig. 2.** Hybridization of 32P transcripts in the presence of competitor transferrin mRNA. Liver nuclei from iron-deficient birds treated with estrogen were allowed to continue transcription in the presence of [α-32P]UTP and the transcripts isolated as described under “Experimental Procedures.” Filter hybridizations were performed with 2.5 × 106 cpm of 32P transcript and 370 cpm of [3H]cRNA as standard in each incubation along with the indicated amounts of nonradioactive transferrin mRNA. The 32P counts per min which hybridized specifically to pBR322-transferrin DNA filters are shown ( ) after subtracting the machine background (18 cpm) and the counts per min bound to wild type filters (~12 cpm). The H counts per min which hybridized are shown ( ) after subtracting machine background (4 cpm) and correcting for spillover of 32P into 3H (1.4%). The pBR322-transferrin filters contained 0.2 μg of plasmid DNA.

### TABLE IV

**The effects of estrogen and iron deficiency on the specific transcription of the transferrin gene**

Liver nuclei were isolated from each group of six birds treated with estrogen and low iron diet as described in the legend to Table II. Nuclei were allowed to continue transcription in vitro in the presence of [α-32P]-UTP and the RNA transcripts were isolated and hybridized to filter bound DNA as described under “Experimental Procedures.”

<table>
<thead>
<tr>
<th>Hormone treatment</th>
<th>Diet</th>
<th>Incorporate UTP</th>
<th>Input to each hybridization</th>
<th>Radioactivity hybridized</th>
<th>Transcription rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pmol/μg DNA</td>
<td>cpm × 10^-6</td>
<td>pBR322 wild type</td>
<td>pBR322 transferrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pCR1 ovalbumin</td>
<td>Transferrin mRNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>corrected*</td>
</tr>
<tr>
<td>No hormone</td>
<td>+Iro</td>
<td>0.48</td>
<td>4.3</td>
<td>9</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>-Iro</td>
<td>0.78</td>
<td>5.34</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Estrogen-withdrawn</td>
<td>+Iro</td>
<td>0.65</td>
<td>5.45</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>-Iro</td>
<td>0.93</td>
<td>5.86</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Estrogen-stimulated</td>
<td>+Iro</td>
<td>0.91</td>
<td>6.0</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>-Iro</td>
<td>1.0</td>
<td>7.03</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

*The efficiency of hybridization, determined using [3H]cRNA, as an internal standard, was 27%; the transcription rates have been corrected to 100% efficiency.
was measured by allowing isolated liver nuclei to continue transcription in the presence of \([\alpha-^{32}\text{P}]\text{UTP}\). Specific transcripts from the transferrin gene then were isolated by hybridization to an immobilized recombinant DNA containing the transferrin cDNA sequence (pBR322-transferrin).

The effects of iron deficiency and estrogen on serum transferrin, transferrin mRNA, and transcription of the transferrin gene in liver are summarized in Table V. Estrogen increases the rate of transferrin mRNA synthesis and the cellular concentration of transferrin mRNA 2- to 2.5-fold but the increase in serum transferrin was only 1.4-fold. In contrast, iron deficiency induced a 1.5-fold increase in transferrin mRNA synthesis, a 2-fold increase in transferrin mRNA levels, and a 4-fold increase in serum transferrin. Since there are many potential variables linking the synthetic rate of transferrin mRNA to the circulating level of serum transferrin, it is not surprising that the relative changes in mRNA synthesis, mRNA concentration, and serum transferrin are not identical. For example, we have measured the rate of transferrin mRNA transcription as a fraction of the total transcription in isolated nuclei; therefore, the observation that the total incorporation in nuclei appeared to increase with either iron deficiency or estrogen as shown in Table IV suggests that changes in the absolute rate of transferrin mRNA production may be greater than our estimates based on relative rates. In addition, hormone or iron-induced changes in the half-life of either transferrin mRNA or circulating transferrin may also play a regulatory role.

The estrogen-mediated increase in liver transferrin mRNA occurs slowly and is associated with gross changes in liver morphology and the synthesis of specific proteins. For example, after 4 days of estrogen treatment, liver weight has increased 2-fold, the rate of albumin synthesis has decreased (3), and the egg yolk protein, vitellogenin, has become the major secretory protein (29). In agreement with the results of Morgan (19), we also find that estrogen causes a dramatic release of liver iron into the serum as shown in Fig. 1 and Table III. Our initial hypothesis was that estrogen and glucocorticoids induce transferrin indirectly by producing acute iron depletion from the liver; however, by administering additional iron to birds receiving these steroids, we were able to maintain elevated liver iron stores without inhibiting the steroid-mediated increase in transferrin mRNA (Table III). An alternative possibility that the iron-deficient response of the transferrin gene is mediated by a stress-related increase in glucocorticoids also seems unlikely. As shown in Table II, iron deficiency does not cause the increase in liver wet weight associated with glucocorticoid administration nor does the level of transferrin mRNA in withdrawn oviduct increase as it would if exogenous glucocorticoids were given at this stage. Furthermore, the synergistic induction of transferrin mRNA in the presence of both estrogen and iron deficiency (Tables II and V) suggests that these inducers are not regulating the transferrin gene through the same mechanisms.

One of the unique features of transferrin gene expression is that two highly differentiated tissues, liver and oviduct, synthesize large amounts of transferrin and that this single copy gene (5) appears to respond to different regulatory signals depending on the tissue environment. Transcription of the transferrin gene in liver is increased 1.5- and 2-fold by iron deficiency and estrogen, respectively. In the oviduct, transferrin mRNA is induced by estrogen but not iron deficiency and the induction by estrogen is mediated by both a >10-fold increase in transferrin gene transcription and an increase in the stability of transferrin mRNA (7).

The estrogen-mediated induction of mRNA synthesis in the oviduct is generally assumed to result from an interaction between the estrogen-receptor complex and chromatin which increases the rate of RNA polymerase initiation at the promoter sites of specific genes. Since liver also has estrogen receptors, a similar mechanism might be evoked to explain the hormonal effects on transferrin mRNA synthesis in this tissue. Very little is known about the interaction of the estrogen-receptor complex and specific genes in oviduct or liver; the observation that protein synthesis is required during the induction in oviduct (23) suggests that regulatory intermediates may be involved. The response of the transferrin gene to iron deficiency in liver must also involve a regulatory signal which is sensitive to some aspect of iron status and can interact, either directly or through other intermediates, with the transferrin gene.

It is important to note that an increase in the rate of transferrin mRNA synthesis does not necessarily imply a change in the rate of RNA polymerase initiation on this gene; an equally plausible explanation is that polymerases which have initiated on the transferrin gene can be terminated prematurely and that this step is regulated by hormones or iron deficiency. Mechanisms involving early termination play an important role in the regulation of several bacterial operons (24–27). An exciting area for future work is the identification of both the regulatory intermediates and the mechanisms by which estrogen and iron deficiency modulate the transferrin gene. A comparison of these regulatory mechanisms in liver and oviduc might also lead to an understanding of how a specific gene is programmed to respond during development and differentiation.

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