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The Ubiquitous Nature of the Progesterone Receptor Binding Factor-1 (RBF-1) in Avian Tissues

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Abstract The avian oviduct receptor binding factor-1 (RBF-1) is a 10 kDa nuclear matrix protein that was originally identified through its ability to effect high affinity interaction of activated progesterone receptor (PR) with chromatin. In the present study, the RBF-1 is shown to not be restricted to reproductive tissues (e.g., oviduct) but present in all avian tissues examined by Western blot analysis with a monoclonal antibody prepared against purified RBF-1. The heart and pancreas had the highest and lowest RBF-1 levels, respectively; the concentration ranging by ~50-fold in these tissues. The 10 kDa size of the RBF-1 detected in all tissues suggests no significant tissue-specific differences in the protein. This was consistent with the finding that purified hepatic and oviductal RBF-1 have identical amino-teminal sequence. Using a recently isolated cDNA to RBF-1, the levels of RBF-1 mRNA were found to correlate well with the ubiquitous presence of the protein as well as tissue-specific differences in concentration. The presence of RBF-1 in non-progesterone responsive tissues suggests the possibility that RBF-1 may not be specifically involved in PR-DNA interactions but may play a more diverse role, possibly involving other steroid receptors such as the glucocorticoid receptor.

Key words: steroid receptor, oviduct, avian tissues, protein, acceptor site

Studies in the last two decades have identified the intricately complex nature of steroid hormone receptor-mediated gene transcription [for a review see Landers and Spelsberg, 1992; Carson-Jurica et al., 1989]. In particular, the study of the interactions of purified steroid receptor complexes with steroid responsive elements (SREs) neighboring steroid-regulated genes have received much attention [for review see Martinez and Wahli, 1991; Beato et al., 1989]. While this interaction is integral to the mediation of transcription, it has recently become clear that other factors, both soluble and chromatin or nuclear matrix-associated, are involved in the alteration of specific gene transcriptional activity. For example, Edwards et al. [1989] have shown that a receptor-associated protein lost during receptor purification is essential for transcriptional activity of the progesterone receptor. Similarly, Diamond et al. [1990] have shown that steroid receptors interact with the nuclear proto-oncoproteins, *Fos* and *Jun* (and perhaps other proteins), at AP-1 transcription factor binding elements to regulate gene expression [for review see Landers and Spelsberg, 1992].

The plethora of literature describing the interaction of steroid receptors with DNA has made it clear that receptors (and their associated factors) are unlikely to interact with DNA alone. The evolving concept that numerous DNA regulatory elements and protein factors are involved for steroid-mediated regulation of gene transcription, make it likely that the nature of the nuclear acceptor sites for steroid receptors in vivo is a complex mosaic of protein and DNA. Nonhistone chromatin proteins and nuclear matrix proteins have been postulated to play a role in steroid receptor-DNA interactions [Spelsberg et al., 1972, 1983, 1984, 1989; Barrack and Coffey, 1980; Barrack, 1987]. However, relatively little is known about the identity and character of these proteins bound to DNA at nuclear acceptor sites, or the role that they play in steroid-mediated gene expression [Rories and Spelsberg, 1989].

The chromatin acceptor sites for the PR have been extensively characterized and found to con-

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sist of complexes of specific acceptor proteins tightly bound to specific DNA sequences [Hora et al., 1986; Spelsberg et al., 1972, 1983, 1984, 1989]. Moreover, chromatin acceptor sites containing chromatin protein-DNA complexes, similar to those described above for the PR avian oviduct system, have been reported for other steroid receptor systems in several species and tissues [Belisle et al., 1986; Chuknyiska et al., 1984, 1985; Cobb and Leavitt, 1987; Crow et al., 1987; *Foekens* et al., 1985; Klyzsejko-Stefanowicz et al., 1976; Kon, 1985; Lopez et al., 1985; Mainwaring et al., 1976; Murray and Towle, 1989; Ogle, 1987; Paulik et al., 1986; Perry and Lopez, 1978; Rennie et al., 1987; Ross and Ruh, 1984; Ruh et al., 1981, 1987; Ruh and Spelsberg, 1982; Shyamala et al., 1986; Singh et al., 1984; Wang, 1978].

Purification of acceptor site proteins for the avian PR has led to the isolation of a small chromatin-associated protein from the oviduct that has been functionally termed the receptor binding factor-1 or RBF-1 [Goldberger and Spelsberg, 1988; Schuchard et al., 1991a,b; Rejman et al., 1991]. Selective removal of the chromatin protein fraction which contains RBF-1 results in the loss of the highest affinity class of PR binding sites. When RBF-1 is reconstituted back onto the avian genomic DNA, the specific binding of PR is regenerated [Spelsberg et al., 1984, 1988; Hora et al., 1986; Goldberger et al., 1987; Schuchard et al., 1991b]. This protein has been purified to apparent homogeneity and shown to be hydrophobic with a $M_r \approx 10$ kDa as determined by SDS-PAGE [Schuchard et al., 1991a,b; Rejman et al., 1991]. Although not completely defined, candidate DNA sequences specific for the binding of RBF-1 have recently been shown to involve upstream regions of the *c-myc* gene including part of exon 1 [Schuchard et al., in press]. Using biochemical analysis and cell fractionation techniques, the RBF-1 has been localized to the nuclear matrix of ovidict tissue [Schuchard et al., 1991a]. Interestingly, this is not only the subnuclear component which binds activated steroid receptors but also the location in which much of the transcriptional machinery of the cell reside [Barrack and Coffey, 1980].

In the present study, we have evaluated the tissue-specificity of the avian RBF-1 using a monoclonal antibody prepared against the purified protein obtained from the oviduct. The finding that RBF-1 can be found in most avian tissues may implicate it as a more general function acceptor protein for steroid receptors.

MATERIALS AND METHODS

Avian Tissues

All tissues were obtained from Pel-Freez Biologicals (Rogers, AR). Briefly, the tissues were removed from the chickens and immediately flash-frozen in liquid nitrogen. They were shipped on dry ice and stored at −80°C until used. Bone cells (cultured giant cells and osteoclasts from freshly harvested chicken bone) were added directly to electrophoresis solubilization buffer and stored (−80°C) until analysed by SDS-PAGE/Western blot with MAb12.

Isolation of Tissue Nuclei

The tissues from 25 animals (ranging from 3–20 g of total tissue) were minced into small pieces with a scissors. A volume of nuclei solution 1 [containing 0.5 M sucrose and 1 × TKM buffer, pH 7.5 (1 × TKM buffer: 0.05 M Tris, 0.025 M KCl, 0.002 M $MgCl_2$, pH 7.5)], 3–4 times the weight was used to blend the tissue in a blender on "high" setting for 10 s, followed by homogenization (5–8 strokes) in a Thomas homogenizer at medium setting. The homogenate was filtered through two layers of cheesecloth to remove debris. The filtrate was centrifuged at 17,000*g* for 5 min at 4°C and the supernatant discarded. The pellets were resuspended in a volume of Nuclei Sol'n 2 (2.0 M sucrose and 1 × TKM buffer, pH 7.5) equivalent to that used with Nuclei Sol 1 with a smooth pestle homogenizer. The concentration of sucrose was adjusted to 1.75 M by the addition of distilled water and the resultant solution centrifuged at 27,000*g* for 10 min at 4°C. The resulting pellet represents partially clean nuclei.

Isolation of Tissue Chromatin From Nuclei

The nuclei pellets were resuspended in the same volume of chromatin solution 3 (containing 0.002 M Tris and 0.0001 M EDTA, pH 7.5) as used with nuclei solutions 1 and 2. Using a smooth pestle homogenizer (by hand), the body of the homogenizer was briskly pulled away from the pestle six or seven times, allowing the vacuum to break the nuclei. The suspension was filtered through four layers of organza (or fine mesh), then centrifuged at 27,000*g* for 10 min at 4°C and the pellets resuspended in a volume of

chromatin solution 4 (0.02 M Tris, pH 7.5) equal to 1–1.5 times the starting tissue weight (v/w). An aliquot for diphenylamine assay was removed to determine DNA concentration. The resulting DNA concentration of the chromatin was 1.0 mg/ml. This was aliquoted and frozen at −80°C until used or extracted with Triton X-100 for preparative purification.

Analytical SDS-PAGE

This was carried out using a 12-well Tris-Tricine gel (1.5 mm thick) with a 10% resolving and a 3.75% stacking gel described previously [Schuchard et al., 1991b]. For analysis of chromatin-associated proteins, 100 μg of chromatin DNA was solubilized in Laemmli's buffer, boiled for 15 min, and electrophoresed for 45 min at 50 V and then for 4 h at 175 V (under liquid cooling). With giant cells or freshly harvested osteoclasts, chromatin was not obtained and the cells were simply added to Laemmli's buffer and directly electrophoresed.

Western Blot Analysis

Samples were analyzed for the presence of RBF-1 by detection with monoclonal antibody 12 (MAb12) using Western immunoblotting as described previously [Schuchard et al., 1991b] with some minor modifications. Briefly, the proteins were blotted to nitrocellulose (for analysis) or ProBlot (for sequencing) in electrode buffer containing 10% methanol, 10 mM CAPS, pH 11, at 100 V (constant current) for 1 h at 4°C. Unbound sites on the nitrocellulose sheets were blocked with 2% milk (w/v; dry stock non-fat) in PBS/0.05% (v/v) Tween 80 for 30 min at 37°C with agitation. The sheets were then incubated in monoclonal antibody 12 (1 μg/ml diluted in 2% milk) for 2 h at 22°C with agitation. After thorough washing with 0.05% (v/v) Tween 80 in PBS, the blot was incubated in alkaline phosphatase-labeled goat anti-mouse IgG diluted 1:1,000 in 2% milk/0.05% Tween 80 for 1 h at 22°C. The blots were again washed and then developed in 0.1 M NaCl and 0.1 M Tris-HCl, pH 9.6, containing 0.1 mg/ml nitroblue tetrazolium, 0.05 mg/ml 5-bromo-4-chloroindoxyl phosphate, and 0.004 M $MgCl_2$ [Blake et al., 1984]. To sharpen the low molecular weight protein bands, the gel system was modified by doubling the Tris-HCl concentration in the resolving gel and in the electrode buffer [Fling and Gregerson, 1986]. The monoclonal antibody detects a 10 kDa protein antigen as described in the previous study with the 10 kDa antigen copurifying with the major PR acceptor activity [Goldberger and Spelsberg, 1988]. Therefore, the 10 kDa antigen which displays a major PR acceptor activity is referred to as Receptor Binding Factor-I (RBF-1). A crude quantitation of RBF-1 in the tissues was carried out with a standard curve which was generated by running pure RBF-1 (50 ng–5 μg) on a 10% gel and scanning the Western blot with reflective densitometry (Shimadzu UV-160).

Purification of Avian Hepatic RBF-1

Triton X-100 extraction of avian hepatic chromatin. A Triton extract of the chromatin obtained from 420 g of avian liver was obtained by homogenizing the chromatin (which is in solution 4) mechanically, using a smooth pestle. The volume was then adjusted to a salt concentration of 0.2 M using 2.0 M KCl. After 5 min on ice, the suspension was centrifuged at 17,000*g* for 10 min. The pellets were then resuspended in solution 5 [0.2% (v/v) Triton X-100 in 1 × NTE, pH 7.5 (NTE = 0.01 M NaCl and 0.01 M Tris and 0.001 M EDTA, pH 7.5)] at a concentration of 0.2 mg/ml (DNA concentration) by hand using a smooth pestle homogenizer. After 2 h of extraction on ice (with occasional stirring), the salt concentration was adjusted to 0.2 M with 2.0 M KCl. After 5 min, the solution was centrifuged at 17,000*g* for 10 min. The 600 ml of (TEC) was concentrated to ≈ 30 ml by ultrafiltration stirred cell (Amicon, Beverly, MA) through a 10 kD cut-off YM-10 membrane while cooled on ice. The 30 ml of liver TEC concentrate was then dialyzed extensively against 6 M GPM buffer (6 M Gdn-HCl, 50 mM phosphate, 1 mM mercaptoethanol; pH 6.0). The final protein concentration was ≈ 8 mg/ml.

FPLC Molecular sieve chromatography. TEC (2 ml; 8 mg/ml) in 6 M GPM was loaded onto a preparative Superose 12 molecular sieve column (Pharmacia, Piscataway, NJ) pre-equilibrated with 6 M GdnHCl. This was accomplished by washing the column with a minimum of 3 bed volumes (150 ml) of buffer at a slow flow rate (1.00 ml/min). Gel filtration separation of the entire 2.0 ml sample was carried out with a flow rate of 1.0 ml/min (backpressure of 0.4 MPa), range set at 0–2.0 absorbance units (AU) and absorbance measured at 280 nm. Twenty-eight 3-ml fractions were collected after 25 ml had eluted from the column. This was repeated

15 times and the fractions from each run, pooled, dialyzed against water, and lyophilized. To identify the fractions containing RBF-1, the lyophilisate from each of the 28 fractions was solubilized in 1.0 ml of distilled water and 10 μl (1/100th of the total) solubilzed in Laemmli's buffer for analytical SDS-PAGE, followed by Western immunostaining.

Preparative SDS-PAGE. A 10% Tris-Tricine gel (1.5 mm thick) was pre-electrophoresed with gluthatione (0.5 mM) for 2 h at 50 mA and then either ran immediately or allowed to stand overnight at 3°C. One tenth of the total molecular sieve purified protein was solubilized in Laemmli's (total volume 1 ml) and loaded into the preparative well of the gel and electrophoresed for 45 min at 50 V and then for 4 h at 175 V (under liquid cooling). The gel was then transferred onto PVDF (4 layered sheets which had been notched on both sides) in ice cold transfer buffer at 100 V for 1 h. The first three sheets were rinsed with water and then stored moist at 4°C while the last sheet was immunostained with the MAb. Upon identification of the location of RBF-1 with the MAb, all four sheets were aligned using the notches as a guide and the band excised. The strip from the three sheets that had not undergone immunostaining were used for sequence analysis.

Amino Acid Sequencing of the Avian Hepatic RBF-1

The band cut from the PVDF containing RBF-I was applied to a gas phase microsequencer for amino acid sequencing by the Edman degradation method. The analyses were performed using an Applied Biosystems 470A protein sequencer by the Molecular Analysis Core Facility. Phenylthiohydantoin amino acids were identified using the Applied Biosystems 120A PTH analyzer. The protein was hydrolyzed with vapors of a 6 N HCl/1% phenol solution in vacuo for 24 h at 110°C.

cDNA for RBF-1

The cDNA for RBF-1 was obtained using polymerase chain reaction procedure. In brief, poly $(A)^+$ mRNA from chicken oviduct was reverse transcribed using an oligo (dT) primer to obtain the first strand cDNA. The cDNA was used as a template in the PCR along with sense primer (oligonucleotides synthesized from the N-terminal sequence of RBF-1) and antisense primer $(dT)_{12-16}$. One of the PCR primers also had a T7 or SP6 phage promoters. The PCR amplified DNA was transcribed and sequenced using RNA amplification with transcript sequencing (RAWTS) method described earlier by Sarkar and Sommer [1988]. When the cDNA was translated, the obtained sequence correlated with the amino acid sequence of RBF-1.

Northern Blot Analysis

Total RNA was isolated from different tissues using a modified method of Chirgwin et al. [1979] as described [Lau et al., 1991]. Poly $(A)^+$ RNA were isolated from total RNA using oligo (dT) column, the mRNA preparations were denatured using glyoxal-DMSO and resolved by electrophoresis in a 1% (v/v) agarose gel [Lau et al., 1991]. The RNAs were transferred overnight to a magna 66 nylon membrane (MSI, Fisher Scientific, Pittsburgh, PA) by capillary action in 20 × SSC (3 M NaCl, 0.3 M trisodium citrate, pH 7.0) as described previously [Lau et al., 1991]. The filters were baked for 2 h at 80°C, hybridized with $[^{32}P]$-labelled cDNA probes, and the blots washed, as previously described [Lau et al., 1991]. The RBF-1 cDNA was labelled with $[^{32}P]$ by radom hexanucleotide primer extension using the Multiprime DNA labelling system from Amersham (Arlington Heights, IL). The $[\alpha\text{-}^{32}P]$ dCTP with specific activity of approximately 3,000 Ci/mmol (New England Nuclear Research Products, Boston, MA) was used to radiolabel the cDNA to achieve specific activities approximately -10^9 cpm/mg.

RESULTS

Tissue Distribution of the RBF-1

Figure 1 shows the results of SDS-PAGE/Western blot analysis of chromatin from the chicken oviduct and liver. While Coomassie staining of the gel showed the expected differences in band patterns of the chromatin protein from oviduct and liver nuclei, Western blot analysis (using the monoclonal antibody) of the same samples shows immunostaining of a 10 kDa band in both preparations. This band is clearly a minor component of the total since the major Coommassie-stained bands between 10–16 kDa represent histones which migrate in this area. Quantitation of the the immunostained bands by scanning reflective densitometry in comparison with a standard curve obtained with known amounts of purified RBF-1, correlated with the presence of 2.7 and 3.4 μg of RBF-1 per 100 μg

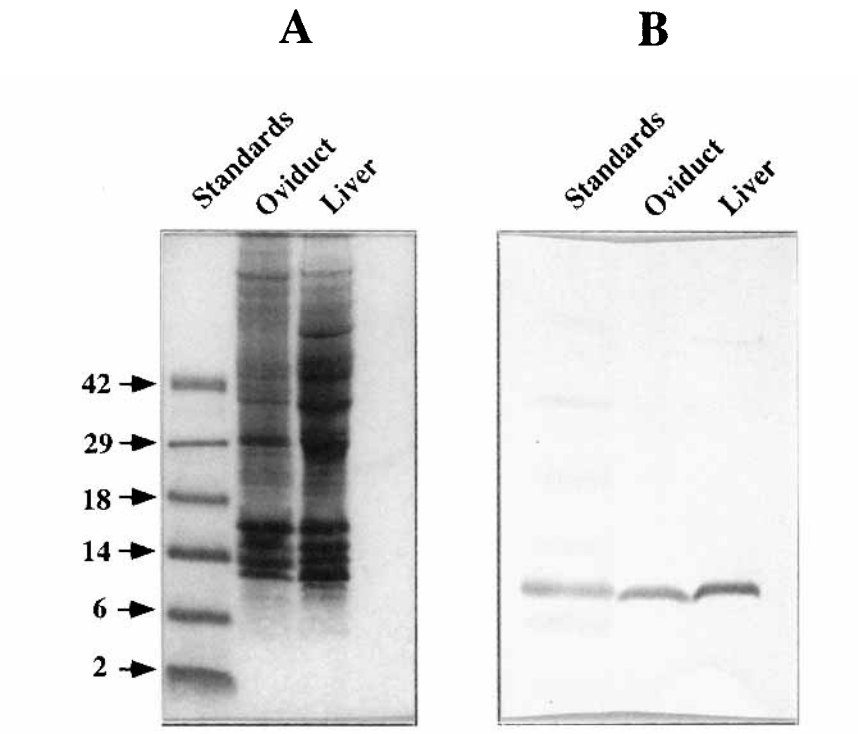

Fig. 1. Denaturing polyacrylamide gel electrophoresis (SDS-PAGE)/Western blot analysis of chromatin from the chicken oviduct and liver. Each lane contains the protein associated with 100 μg of chromatin DNA. The standard lane in both panels contains 1 μg of purified RBF-1 (in addition to the molecular weight standards) as a positive control for the Western blot.

DNA for oviduct and liver, respectively. This roughly represents approximately 100,000 molecules per cell [Schuchard et al., 1991b]. Similar analysis of the chromatin isolated from other avian tissues is shown in Figure 2A which indicates that RBF-1 is present in a diverse array of tissues including the heart, lung, brain, kidney, and pancreas. The tissue-dependent variation in RBF-1 concentration is best illustrated by the bar graph given in Figure 2B. RBF-1 is present in the highest concentration in heart chromatin and lowest in pancreatic tissue, with a difference in concentration between the two of ≈ 50-fold. A qualitative evaluation of the presence of RBF-1 in bones cells was accomplished using cultured giant cells and osteoclasts obtained from freshly harvested chicken bones [Oursler and Osdoby, 1988; Oursler et al., 1991]. The Western blot in Figure 3 shows the presence of an immunoreactive protein, in both tissues, that co-migrates with the 10 kDa RBF-1 from the oviduct. The significance of a decreased cellular concentration of the RBF-1 when giant cells are cultured in a conditioned media is not clear at the present time.

Purification and Partial Characterization of Hepatic RBF-1

To determine whether there were tissue-specific differences in the RBF-1 protein within the same species, RBF-1 from hepatic tissue was purified. This tissue was chosen over cardiac tissue, which possesses the highest RBF-1 concentration (per μg DNA), due to (1) the size of the organ (≈ 20 g per animal), (2) its relatively high RBF-1 content, and (3) the ease with which this tissue is homogenized (i.e., no connective tissue). Hepatic RBF-1 was purified through a combination of centrifugation, Triton X-100 extraction of the chromatin, FPLC molecular sieve chromatography, and preparative SDS-PAGE/Western blotting, and final product analyzed for amino-terminal sequence.

The nuclei and chromatin were isolated as described in the Materials and Methods section. A Triton X-100 extract of chromatin (TEC) was obtained with 0.2% (v/v) Triton X-100 which typically extracts ≈ 20% of the chromatin bound protein. The lyophilisate (obtained after dialysis

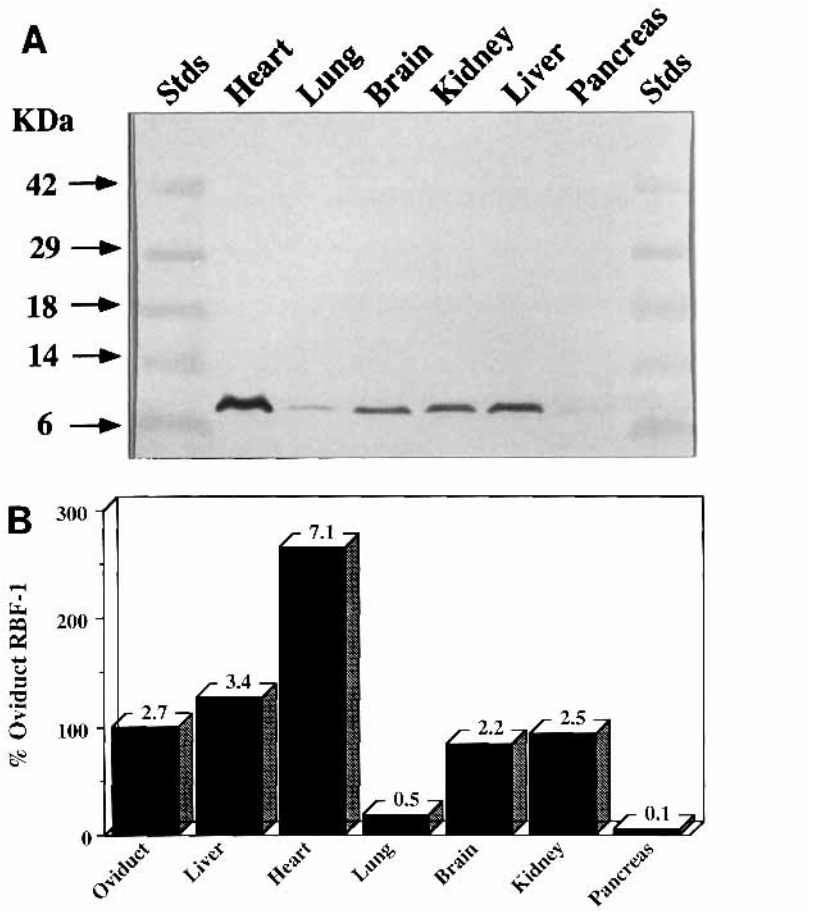

Fig. 2. Western blot detection of RBF-1 in other avian tissues. Each lane run on SDS-PAGE contains the protein associated with 100 μg DNA from the chromatin isolated from each of the respective tissues. **A:** The Western blot. **B:** The tissue-dependent variation in RBF-1 concentration. Values at the top of each bar represent μg RBF-1/100 μg DNA.

against 2 M urea and water) was solubilized in a buffer containing 6 M guanidine hydrochloride and chromatographed on a preparative FPLC molecular sieve column. The presence of RBF-1 in the collected fractions was determined by SDS-PAGE followed by Western blot analysis (Fig. 4). Figure 4A shows the profiles for the presence of protein (absorbance at 280 nm; solid line) and, specifically, RBF-1 (intensity of immunostaining; dotted line) in each fraction. Elution of RBF-1 in the 65–75 ml range was consistent with a protein having a low molecular mass (i.e., < 50 kDa). Preparative SDS-PAGE was carried out with the protein obtained in the pooled fractions (65–75 ml), followed by transfer to PVDF, isolation, and extraction of the protein with isopropanol/TFA which resulted in a pure RBF-1 preparation. Figure 4B illustrates the relative protein composition of molecular sieve purified TEC and the preparative SDS-PAGE purified RBF-1.

The sequence of the 10 N-terminal amino acids of the purified hepatic RBF-1 was to be identical to the M-I-P-P-V-Q-V-S-P-L sequence previously determined and reported for the avian oviduct RBF-1 [Rejman et al., 1991].

Detection of RBF-1 mRNA

A cDNA for RBF-1 was obtained by using the polymerase chain reaction (PCR) procedure. Northern blot analyses were performed on selected tissues to determine if RBF-1 mRNA levels correlated with the tissue distribution of the protein. RBF-1 mRNA was observed in all of the tissues (oviduct, liver, heart, kidney, and brain) with the highest levels found in heart and liver (Fig. 5). The actin gene was used as a control to show that the observed variations in RBF-1

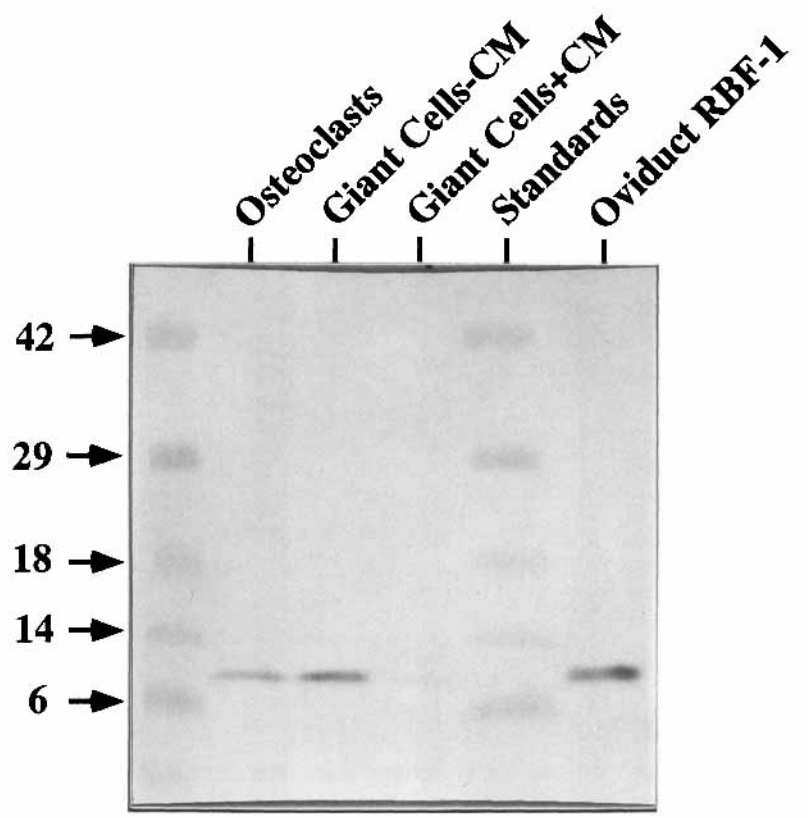

Fig. 3. Western blot detection of RBF-1 in avian bone cells. The RBF-1 in chicken giant cells and osteoclasts comigrates with the RBF-1 from the oviduct. CM represents conditioned media.

mRNA levels were not the result of uneven loading. The rank order for levels of mRNA in each of these tissues correlated well with that observed at the protein level.

DISCUSSION

Relatively little is known of the identity and character of the nuclear proteins bound to DNA at nuclear acceptor (binding) sites and the role that they play in steroid-mediated gene transcription [Rories and Spelsberg, 1989]. However, the abundant literature on acceptor sites is testament of their importance in steroid-mediated events. The chromatin acceptor sites for the PR have been extensively characterized in several tissues and found to consist of complexes of specific acceptor proteins tightly bound to specific DNA sequences [Hora et al., 1986a; Spelsberg et al., 1972, 1983, 1984, 1989; Perry and Lopez, 1978; Cobb and Leavitt, 1987]. Furthermore, chromatin acceptor sites containing chromatin protein-DNA complexes similar to those described above for the PR-avian oviduct system, have been reported for progesterone receptors (PR) in rat placenta [Ogle, 1987], hamster uterus [Cobb and Leavitt, 1987], and sheep hypothalmus [Perry and Lopez, 1978]; for the estrogen receptors (ER) in the avian oviduct [Ruh and Spelsberg, 1982], cow and rabbit uteri [Ruh et al., 1981; Ross and Ruh, 1984; Singh et al., 1984], rat [Chuknyiska et al., 1984, 1985] and mouse [Pavlik et al., 1986] uteri, human and animal breast cancer cells [Shyamala et al., 1986], and in sheep hypothalamus [Perry and Lopez, 1978; Lopez et al., 1985]; for androgen receptors in rat prostate and sertoli cells [Klyzsejko-Stefanowicz et al., 1976; Wang, 1978; Mainwaring et al., 1976; Foekens et al., 1985; Rennie et al., 1987]; for glucocorticoid receptors [Ruh et al., 1987]; and finally, for thyroid hormone receptors [Murray and Towle, 1989]. Specific chromatin acceptor sites involving acceptor proteins for the retinol receptor in rat liver have also been shown [Crow et al., 1987]. Several laboratories have also studied the differences in chromatin acceptor sites for antiestrogen- vs. estrogen-bound receptors [Kon, 1985], as well as for the estrogen and progesterone receptors

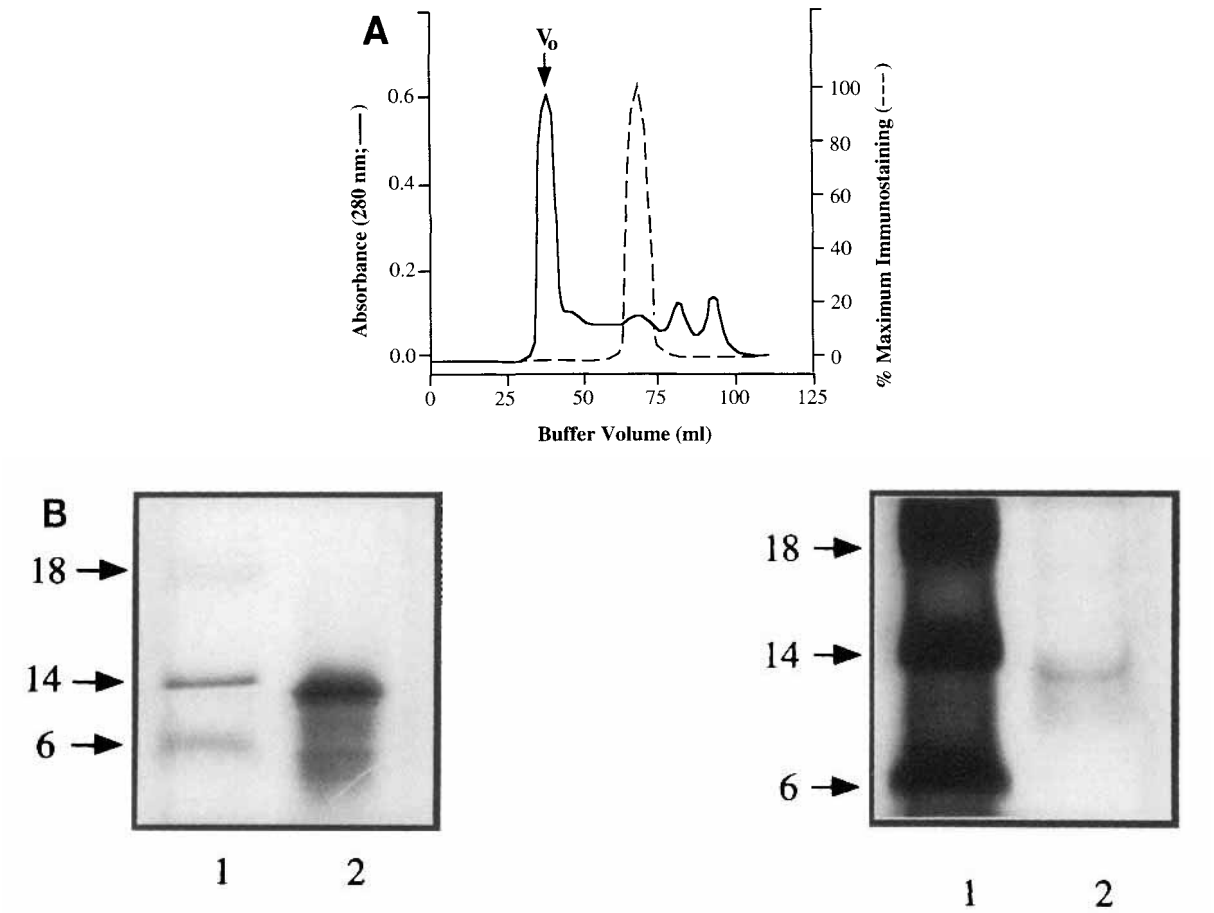

Fig. 4. FPLC purification of avian hepatic RBF-1. **A:** Absorbance and immunostaining profile of fractions from the molecular sieve chromatography of a 0.2% Triton X-100 extract of hepatic chromatin. The presence of RBF-1 in collected fractions was determined by Western blot analysis with the MAb after extensive dialysis and lyophilization. **B:** PAGE analysis of the protein resulting from: molecular sieve purification of TEC (left; Coomassie-stained) and pure RBF-1 from preparative SDS-PAGE (right; silver-stained).

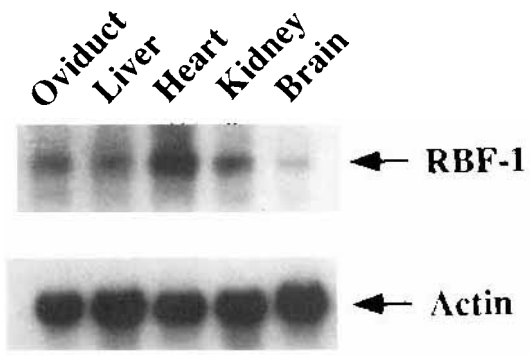

Fig. 5. Detection of RBF-1 mRNA in various avian tissues. Actin gene was probed as a control for equal RNA loading.

during hormone-induced sexual differentiation mediated by the hypothalamus [Perry and Lopez, 1978; Lopez et al., 1985]. Age-related changes in nuclear acceptor sites for ER in rat uterus [Chuknyiska et al., 1985] and murine pituitary [Belisle et al., 1986] have also been reported.

Although not completely understood, the importance of other non-receptor proteins in steroid-mediated events has been revealed. These studies include non-receptor proteins in the PR system of the avian oviduct [Bagchi et al., 1988] and in the tissues of other animal species [Edwards et al., 1989], as well as glucocorticoid receptors in rat liver [Okamoto et al., 1988]. Interestingly, Ruh and coworkers [Ross and Ruh, 1984] have identified "acceptor proteins" for the estrogen receptors in the rabbit and cow uteri and found a striking structural similarity to the RBF-1 described by our group for the avain oviduct [Goldberger and Spelsberg, 1988; Schuchard et al., 1991b; Rejman et al., 1991]. The fact that these proteins are small, hydrophobic, tightly bound to DNA proteins, and similar in size (but do not cross-react with anti-RBF-1 mono or polyclonal antibodies) may indicate a "family of structurally similar acceptor

proteins." One of these proteins from the rabbit uterus, a 16 kDa acidic protein with properties similar to those of RBF-1, has recently been purified (Ruh, personal communication). Finally, a 20 kDa acceptor protein bound by the rat prostate androgen receptor in vivo was identified by crosslinking the receptor to its nuclear/chromatin acceptor sites [Rennie et al., 1987]. About 80% of the AR was reportedly bound to this protein while 20% of the AR was bound to DNA in this system [Foekens et al., 1985]. An acceptor-like protein has also been identified for binding the thyroid hormone receptors to the genomic DNA [Murray and Towle, 1989].

A recent study using immunohistochemical techniques has shown that the RBF-1 is not only localized to the nucleus but present only in those cells known to contain PR (epithelial) [Zhuang et al., 1993]. The immunohistochemical localization of RBF-1 confirms the results obtained with cruder localization approaches showing this protein to be primarily localized to the nuclei of cells [Schuchard et al., 1991a; Goldberger et al., 1987; Spelsberg et al., 1984; Hora et al., 1986]. Localization of both RBF-1 and PR to the nuclear interior is consistent with the observations that both steroid receptors and the nuclear acceptor proteins have been localized in the nuclear matrix [Barrack, 1987; Schuchard, 1991a]. This is an important observation in light of the growing realization that the nuclear matrix is an important structure in gene regulation [Barrack and Coffey, 1980].

All of the early studies on RBF-1 utilized avian oviduct tissue as the model system [Hora et al., 1986; Spelsberg et al., 1972, 1983, 1984, 1988, 1989; Goldberger and Spelsberg, 1988; Rejman et al., 1991; Goldberger et al., 1987]. Since RBF-1 was originally shown to be specific for activated PR binding to DNA, the avian oviduct has been an ideal progesterone-regulated tissue system to study. Schuchard et al. [1991b] were the first to identify the presence of RBF-1 in extra-oviductal, non-progesterone regulated tissues. Using an MAb generated against purified oviduct RBF-1, 10 kDa immunoreactive proteins were found in avian liver and spleen. The present study confirms these findings and demonstrates that RBF-1 is not restricted to the oviduct, liver, and spleen in the avian system, but can be found in varying concentrations in most tissues. The presence of RBF-1 in bone tissue is particularly interesting. The strong estrogen-dependent integrity of bone has been clearly linked to the action of osteoblasts (bone building) and osteoclasts (bone breakdown) [Oursler et al., 1993]. The recent discovery of estrogen receptors in osteoblasts [Eriksen et al., 1988] and osteoclasts [Oursler et al., 1991] confirms that these are, indeed, estrogen target tissues, again presenting the possibility that RBF-1 may be involved in regulation of gene expression and cellular activity.

The immunochemical detection of RBF-1-like antigens in other avian tissues is most intriguing in light of the fact that most of these tissues are not considered to be responsive to progesterone. While there appears to be a lack of tissue-dependent differences with respect to the presence of RBF-1, there certainly appears to be a tissue-dependence in the concentration of RBF-1. The $\approx$ 50-fold difference in RBF-1 concentration between the heart and pancreas may be providing some, as yet undetermined, clues to its function. The cell-type specific localization of RBF-1 [Zhuang et al., 1993] may be relevant to this observation.

The ubiquitous nature of the RBF-1 in chicken tissues may also be revealing a somewhat broader function served by this nuclear matrix protein. These may include functioning in non-receptor processes or serving analogous functions for other steroid receptors in other species. This is supported by the similarity of the glucocorticoid and progesterone receptor systems (including similarity of their regulatory elements) and the glucocorticoid-responsive nature of tissues such as the heart and liver. This presents the possibility for the existence of an RBF-1 "class of proteins" which serve acceptor functions for both progesterone and glucocorticoid receptors.

In conclusion, the RBF-1 of the PR avian oviduct system may be one of several such proteins involved in the nuclear acceptor sites for the avian oviduct progesterone receptor. Earlier studies evaluating the binding activities of fractions from hydroxylapatite, HPLC-reverse phase, and HPLC-molecular sieve chromatographies revealed the possible existence of other (non-10 kDa proteins) PR acceptor activities. Whether these proteins play an active role at the same sites or are represented as distinct acceptor sites remains to be determined. Clearly, the studies currently underway on the biological function of the RBF-1 protein using overexpression and antisense technologies will be of intense interest.

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