Control of Transcription of the Chicken Progesterone Receptor Gene

IN VITRO AND IN VIVO STUDIES*

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To study the promoter of the chicken progesterone receptor (cPR) gene and the relevance of several progesterin-responsive elements therein, chimeric genes were constructed which contained the 5′-flanking region of the cPR gene linked to promoterless globin or chloramphenicol acetyltransferase sequences. Cell-specific initiation of transcription was observed in transiently transfected chicken embryo fibroblasts when using 876 base pairs of the cPR gene upstream region. Transcription from these reporter genes could be induced by progesterins in the presence of cPR form A but not of form B. In keeping with these data, three in vitro progesterone receptor (PR)-binding sites were identified in the cPR promoter region by DNase I protection assays. However, in vivo, nuclear run-on transcription demonstrated that neither primary stimulation with progesterins, nor treatment of secondarily estrogen-stimulated chicks with progesterins, glucocorticoids, or androgens resulted in any significant change of cPR gene transcription in the oviduct, thus suggesting a cell- and/or development-specific role for these progesterin-responsive elements. Although estrogen is known to increase PR levels in the chick oviduct, this effect does not involve stimulation of PR gene transcription, as demonstrated here by nuclear run-on experiments, the analysis of DNase I hypersensitive sites, and transient cotransfection studies. Since acute withdrawal from estrogen-stimulation markedly decreased the level of cPR mRNAs in chick oviduct when analyzed by Northern blotting, we conclude that estrogen-dependent stimulation of PR levels in the oviduct is a post-transcriptional process.

Steroid hormone receptors belong to a superfamily of transcriptional regulatory proteins which, in response to binding of their cognate ligands, specifically recognize DNA sequences of target genes, resulting in the coordinate expression of gene networks. The cloning of cDNAs encoding steroid hormone receptors and subsequent structure-function analyses has permitted the identification of receptor domains responsible for DNA binding (DBD), hormone binding (region E) (for review, see Green and Chambon, 1988; Evans, 1988) and dimerization (Kumar et al., 1988). Evidence has been presented for the existence of two transcription activation functions, one within the N-terminal region A/B and the other within the hormone-binding domains of the human estrogen, glucocorticoid, and progesterone receptor (Webster et al., 1988; Bocquel et al., 1989; Meyer et al., 1990; Tora et al., 1990a).

Little is known about mechanisms controlling steroid hormone receptor synthesis. For the PR, it has been shown that estrogen increases PR levels both in mammals (Milgrom et al., 1973; Loosfelt et al., 1984) and chicken (Toft and O'Malley, 1972; Mester and Baulieu, 1977; Moen and Palmiter, 1980), and that estrogen induces PR mRNA in human MCF-7 breast cancer cells (Read et al., 1988; Narahalli et al., 1988). Autoregulation of PR has also been reported. In mammals, progesterins down-regulate their own receptors (Milgrom et al., 1973; Wei et al., 1988), while in the non-differentiated chick oviduct, progesterone increases the PR content (Ylikomi et al., 1984). Thus, it appears that expression of cPR in chick oviduct is under complex hormonal control and may be linked to the state of differentiation of this organ. Oviduct differentiation is itself induced and maintained in sexually immature female chicks following administration of steroid hormones. The initial induction (primary stimulation) requires estrogen and results in differentiation and proliferation of tubular gland cells located in the magnum portion of the oviduct and in the synthesis of egg-white proteins (for reviews and Refs. see, Schimke et al., 1973; Palmiter et al., 1978). When administration of estrogen is halted (withdrawal), the oviduct regresses and production of egg-white protein ceases. However, production can be reinduced (secondary stimulation) in the remaining tubular gland cells by both estrogen and nonestrogenic steroid hormones (Shepherd et al., 1980 and references therein).

Two forms of cPR (form A (M, = 79,000) and form B (M, 1 The abbreviations used are: DBD, DNA-binding domain; PR, progesterone receptor; cPR, chicken progesterone receptor; PRE, progesterone responsive element; GRE, glucocorticoid responsive element; CAT, chloramphenicol acetyltransferase; CEF, chicken embryonic fibroblasts; DHEA, Dione 1 hypersensitive site; bp, base pair; kb, kilobase; DES, diethylstilbestrol; RU486, promegestone, 17a,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione; RU486, RU38486, 17a-hydroxy-11β-(4-dimethylaminophenyl)-17a-(1-propynyl)-estradiol-4,9-dien-3-one; 9a-fluoro-11β,17α,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione; TA, trimaminolone acetone, 9α-fluoro-11β,16α,17β-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetol with acetone.
present in pBLCAT8+ and recircularization of the BarnHI/BglII containing the rabbit 8-globin sequences from position -9 to about al.,
coding sequences of the pPR plasmids were removed by SmaI diges-
EamHI site was introduced at position -137 and the corresponding
probe encompassing exon 1 of the cPR gene (Jeltsch et al., 1990) was
hybridization to filter-bound DNA, and washing was performed as
moter apparently contains progestin- but no estrogen-responsive
elements. These data are compared with the in vivo regulation of PR gene expression in chick oviduct as assayed by
Nuclear Run-on Assays—Nuclei from oviducts were isolated as described (Kaye et al., 1986) and stored at -80 °C in 20 mM Tris-
DNA Footprinting—DNase I protection assays were performed according to Eul et al. (1989) using the affinity purified bacterially
RESULTS
Faithful Initiation of Transcription from the cPR Promoter in Transiently Transfected Chicken Embryonic Fibroblast Cells—In order to provide evidence that the recently described 5'-flanking region of cPR (Jeltsch et al., 1990) in fact contains the PR promoter, we have examined whether transcription initiation from this promoter could be demonstrated by transient transfection of chimeric genes. For this, a construct containing PR gene sequences from -867 to +77 bp linked to the β-globin gene was transfected into chicken embryonic fibroblast cells (CEF), and RNA was isolated for S1 nuclease analysis. Using an end-labeled single-stranded probe, generated by primer extension of oligonucleotide M6 (schematically illustrated in Fig. 1B), a major protected fragment of 146 nucleotides was obtained (Fig. 1A, lane 1), indicating initiation of transcription at the site previously described for oviduct laying hen RNA (indicated by +1 in the sequence shown in Fig. 1A, note that the signal at 60 nucleotides originates from the cotransfected internal control recombinant pG1B (Gronemeyer et al., 1987). Other sites of initiation, close to +1, can be seen as well (Fig. 1A, lane 1). Protection was specific for the PR promoter, since none of the fragments was apparent when pBSM13+ (Stratagene) was transfected instead of the chimeric gene (lane 2), and only the 60 nucleotide long fragment obtained by protection of RNA transcripts from the cotransfected pG1B was seen. A similar activity of the PR promoter was observed when constructs containing up to 4.1 kb of PR gene sequences were transfected into CEF (data not shown).

The PR Gene Promoter Is Responsive to Progestins and Glucocorticoids When Transfected into CEF Cells—In order to investigate whether the hormone inducibility of PR expression (as introduced) is due to the presence in the cPR promoter of a corresponding steroid-response element, chimeric reporter genes were constructed in which cPR gene sequences from -1683 to +80 were linked to the promoterless chloramphenicol acetyltransferase (CAT) gene (using a parent vector pBLCAT8+ in which the tk sequences were removed; see Klein-Hitpass et al., 1986) yielding (−1683/ +80)CAT (illustrated in Fig. 1C). Truncation of 5’ and subsequent 3’ PR sequences of (−1683/80)CAT generated (−876/80)CAT and (−876/137)CAT, respectively (Fig. 1C). The presence of DNA elements responsive to hormones within these PR sequences was analyzed by transient transfection into CEF of these reporter plasmids together with vectors that express the various steroid hormone receptors. In order to “correct” for variations in transfection efficiencies, data were normalized based on the activity obtained from the internal control recombinant pCH110, which expresses β-galactosidase (Gronemeyer et al., 1987). Cotransfection of (−1683/80)CAT and 1-5 μg of the vector cPR2 (expressing cPR form A; Gronemeyer et al., 1987) resulted in a 3-5-fold
FIG. 1. The PR gene promoter is responsive to progesterone or glucocorticoid when transfected into CEF cells. A, S1 nuclease mapping of cytoplasmic RNA isolated from CEF cells transfected with 5 µg of plasmid pPR-GLOB (illustrated in B) and 0.2 µg of pG1B (lane 1) or 5 µg of plasmid pBSM13+ (Stratagene) and 0.2 µg of pG1B (lane 2). GATC is a sequence ladder obtained by the Sanger dideoxy method using primer M6 (Zenke et al., 1986) which was also used for probe synthesis. The solid arrow (+1) indicates within the corresponding sequence (left, compare sequence ladder) the cap site as mapped with RNA isolated from laying hen oviduct (Gronemeyer et al., 1987); arrowheads indicate the position of protected fragments corresponding to RNA initiated at the globin cap site in pG1B (60 nucleotides) or at the PR cap site in pPR-GLOB (146 nucleotides). B, schematic illustration of S1 nuclease mapping analysis. PR and globin sequences of pPR-GLOB are indicated. M6 is the primer used for probe synthesis, an NsiI site at -709 was used to generate the probe. C, structure of cPR promoter-chimeric recombinants (-1683/+80)CAT to (-867/-137)tk-CAT (for details of construction, see “Materials and Methods”). The arrow indicates the transcriptional start site. tk refers to the Herpes simplex thymidine kinase promoter. D, CAT assays using extracts of chicken embryo fibroblast cells transfected with 5 µg of reporter plasmid (0.5 µg for (-867/-137)tk-CAT), 1 µg of expression vector for either the cPR form B (cPR1), form A (cPR2), human glucocorticoid receptor (HGR; Kumar et al., 1987), or chicken estrogen receptor (CEO; Tora et al., 1989b), and 2 µg of the internal control recombinant pCH110 (Gronemeyer et al., 1987). Hormones R5020 (R), estradiol (E), or triamcinolone acetonide (TA) were used at a final concentration of 20 nM. CAT activity was determined by counting 14C in excised spots from thin layer chromatographs and subtracting the background (except for lanes 8 and 9) obtained with the promoterless vector pBLCAT(-tk) (see “Materials and Methods”). E, dose-response curve demonstrating inducibility of (-1683/+80)CAT by cPR form B (cPR1), form A (cPR2), human glucocorticoid receptor (HGR; Kumar et al., 1987), or chicken estrogen receptor (CEO; Tora et al., 1989b), and 2 µg of the internal control recombinant pCH110 (Gronemeyer et al., 1987). Hormones R5020 (R), estradiol (E), or triamcinolone acetonide (TA) were used at a final concentration of 20 nM. CAT activity was determined by counting 14C in excised spots from thin layer chromatographs and subtracting the background (except for lanes 8 and 9) obtained with the promoterless vector pBLCAT(-tk) (see “Materials and Methods”). 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induction of CAT activity when the synthetic progesterin R5020 (R) was added (Fig. 1D, compare lanes 2 and 3; 1E, lanes 8–13). This induction was not observed when cPR2 was replaced by its parental vector, pKCR2 (Breathnach and Harris, 1983) (Fig. 1E, lanes 6 and 7). Interestingly, no activation of (-1683/+80)CAT was seen with 1-5 μg of cPR1 (Gronemeyer et al., 1987) which generates cPR form B (Fig. 1D, lanes 4 and 5; E, lanes 1–5). The absence of activation of the cPR promoter by cPR form B was not due to inefficient or aberrant expression of this receptor protein, since both forms A and B were expressed at similar levels as confirmed by immunoblot analysis (data not shown, see also Kastner et al., 1990). Deletion of PR gene sequences from -1683 to -867 bp yielded a reporter gene ((-876/+80)CAT) displaying about 1.5-3.5-fold induction by cPR form A (0.5-5 μg of cPR2 were used; Fig. 1D, compare lanes 6 and 7; F, lanes 13–20). Note that this deletion did not alter the differential activation of transcription by the two cPR isoforms of the reporter recombinant (cPR form B is inactive, Fig. 1F, lanes 3–10). Sequences upstream of -1683 apparently do not contain further promoter elements which are active in CEF, since transfection of constructs with PR gene sequences up to -4.1 kb were similarly induced by progesterin/cPR2 as (-1683/+80)CAT (data not shown). Deletion in (-876/+80)CAT of the PR sequences from -137 to +80 resulted in a reporter gene ((-876/-137)CAT) which showed only background activity, indicating the presence of essential promoter elements in this region of the cPR gene (Fig. 1D, lane 10; see also Jeltsch et al., 1990). (-1683/+80)CAT was also activated by triamcinolone acetonide (TA, a synthetic glucocorticoid) when the glucocorticoid receptor expression vector HGO (Kumar et al., 1987) was cotransfected (lanes 13 and 14), albeit to a lesser extent than by cPR form A. Progesterone/cPR2 (but not cPR1) inducibility was conferred onto a heterologous promoter when PR gene sequences from -867 to -137 were inserted in front of the thymidine kinase (tk) promoter in pBLCAT8 to give (-876/-137)tk-CAT (Fig. 1, C and D, lanes 8 and 9). Note that 10 times less (-876/-137)tk-CAT was used in lanes 8 and 9 as compared with reporter genes lacking the tk promoter (lanes 1–7 and 10–14). As expected no progesterin/cPR2 inducibility was observed when the parental recombinant pBLCAT8′ (Fig. 1C) was used in place of (-867/+80)tk-CAT (data not shown). It is important to note that, when transfected into MCF-7, HeLa, or HepG2 cells (-1683/+80)CAT and (-867/+80)CAT were not constitutively active, nor could they be induced by cPR form A or B (data not shown), thus suggesting that a cell-specific factor(s) is required for PR promoter activity.

The PR Promoter Contains PR/Glucocorticoid Receptor-binding Sites—The activation of the (-1683/+80)CAT reporter gene by cPR form A prompted us to look for progesterone/glucocorticoid receptor binding in the cPR promoter region. As reported previously, the sequence TGT(T/C)CT, which is the most conserved motif among various PRE/GREs (reviewed in Beato et al., 1989), was found at positions -246, -890, -908, -989, and -1113 bp relative to the cap site (Jeltsch et al., 1990; see also Fig. 2). To investigate whether these motifs are able to bind PR in vitro, we used a bacterially expressed cPR DNA-binding domain fused to β-galactosidase (cPR-FP3; see Eul et al., 1989) in DNase I protection experiments. A clear footprint covering the entire promoter proximal motif was observed (Fig. 2, compare lane 1 with lanes 2–4). The binding of cPR-FP3 also resulted in the appearance of hypersensitive sites, for example at position -209. Two further protected regions were mapped between positions -916 and -870 (compare lanes 6 and 7). Note, however, that four times more fusion protein was required to obtain weak footprints in lane 7 as compared with the one shown in lane 2. No protection was seen for the potential PRE/GRE at -984 to -998 even when analyzed with fragments which were end labeled at more proximal restriction sites (positions -940 or -1066) (data not shown). No footprints were detected for the most 5′ potential PRE/GRE (at -1113) when labeling the HindIII site at position -1063 and using high amounts of fusion protein (approximately 1 μg, data not shown). In conclusion, only one of the five candidate PRE/GREs, that is closest to the transcriptional start site of the cPR gene, is able to strongly bind the PR DNA-binding domain in vitro. Two motifs further upstream are only weak binders, while the most distal ones do not interact with the PR fusion protein in vitro.

The Oviduct PR Gene Transcription Is Not Affected by Progesterone, Glucocorticoid, or Testosterone Treatment in Vivo—The transient cotransfection experiments with PR promoter chimeras and cPR2 and the in vitro footprinting analyses described above suggested to us the existence of functional progesterone/glucocorticoid-responsive elements in the PR promoter. In keeping with these data, it has been reported that primary stimulation by progesterone increases the PR level in the non-differentiated oviduct (Ylikomi et al., 1984). These observations prompted us to look for changes in PR gene transcription upon progesterone treatment. Ten-day-old chicks were subjected to primary stimulation with progesterone for 2 weeks, and oviduct nuclei were isolated for run-on experiments as described under “Materials and Methods.”
This treatment, however, does not modify PR gene transcription with respect to untreated animals (Fig. 3A, compare lanes 1 and 2). This is also true when progesterone was given to chicks secondarily stimulated with DES (Fig. 3A, compare lane 3 with lanes 4 and 5) or withdrawn from secondary estrogen stimulation for 1 day before progesterone injection (Fig. 3A, compare lane 6 with lanes 7 and 8). The increase observed for ovalbumin or Y gene transcription (lanes 6–8) confirms that progesterone treatment was effective (McKnight and Palmiter, 1979; LeMeur et al., 1981). Since it is known that glucocorticoid and androgen receptors recognize the same DNA-responsive element as PR (Ham et al., 1988 and references therein), we also examined the effect on cPR gene transcription of the corresponding hormones. Secondarily, estrogen-stimulated chicks were withdrawn for 2 days and treated for 12 h with either dihydrotestosterone (T) or dexamethasone (D). None of these hormones affected PR gene transcription (Fig. 3B, compare lane 1 with lanes 3, 4, or lane 2 which corresponds to progesterone (P) treatment). Again, the efficiency of hormonal treatments is shown by the increased transcription of the ovalbumin gene (Fig. 3B, compare lane 1 with lanes 2–4) which is known to be regulated by both dexamethasone and dihydrotestosterone (Hager et al., 1980; Compere et al., 1981). Also RU486 which is an antigu- locorticoid but not antiprogestin in chicks (Baulieu, 1985) was without effect in these assays (Fig. 3B, lane 5). Thus, in oviduct nuclei under the conditions described above, no change in PR gene transcription following progesterin, glucocorticoid or antiglucocorticoid, or androgen administration could be detected.

Estrogen Does Not Regulate PR Gene Transcription, but Estrogen Treatment Increases PR mRNA Levels in the Chick Oviduct. In the chick oviduct, the PR level is under estrogen control, being positively regulated upon primary stimulation and negatively upon withdrawal from secondary estrogen treatment (Toft and O'Malley, 1972; Moen and Palmiter, 1980; Mester and Baulieu, 1977). In order to analyze whether these changes correlated with variations of the cPR mRNA level, Northern blot analyses were performed with oviduct poly(A)⁺ RNA isolated from chicks secondarily stimulated with DES and then either withdrawn or maintained on hormone for 3 days (for details see legend to Fig. 4 and “Materials and Methods”). Using a cPR cDNA probe, we observed a decrease in the level of all cPR mRNA species after DES withdrawal (Fig. 4, compare lanes S and W and data not shown). As expected, the ovalbumin mRNA level, which is estrogen-regulated (McKnight and Palmiter, 1979) is also strongly diminished in RNA from withdrawn chicks (Fig. 4) while hybridization of the same blot with an actin probe confirms that equivalent amounts of poly(A)⁺ RNA were applied in both lanes (Fig. 4, bottom). These results suggest that estrogen is required either for stimulation of PR gene transcription or for an increase in PR mRNA levels due to estrogen-dependent post-transcriptional phenomena.

To investigate whether PR regulation by DES occurs at the translational level, run-on assays were performed using a probe complementary to the first intron of the cPR gene (see “Materials and Methods” and Jeltsch et al., 1990) and oviduct nuclei from chicks treated as described above. When normalized according to the signals obtained by hybridization to an actin probe, similar rates of PR gene transcription were observed for oviduct nuclei from stimulated (Fig. 3A, lanes 3 and 9), and 1- or 3-day withdrawn (lanes 6 and 10, respectively) chicks. Controls with ovalbumin or Y gene probes (LeMeur et al., 1981) confirmed that hormonal withdrawal was effective (compare lanes 3, 9 with lanes 6, 10), since transcription of these estrogen-induced genes is diminished in nuclei from withdrawn chicks, relative to the signals obtained for actin. The hybridization seen with the PR probe was strand-specific since a probe of the opposite strand PR(-strand) (Fig. 3) gave a background signal. In addition, the PR probe does not contain any repetitive sequences since it hybridized to a single band on a Southern blot (data not shown). Moreover, the signals are RNA polymerase B dependent, since addition of 1 [microg]/ml a-amanitin to the elongation mix gave no hybridization (data not shown).

In agreement with these results, we did not detect any estrogen-dependent stimulation of cPR promoter-chimeric reporter recombinants in transient transfection experiments: no increase of CAT activity was noted when CEF cells co-transfected with the chicken estrogen receptor expression vector (Tora et al., 1989b) and (-1683/+480)CAT were exposed

**Fig. 3.** Effect of hormonal treatment on PR, ovalbumin, and Y gene transcription. A, 10-day-old chicks were subjected to various hormonal regimens (P, progesterone) as primary (lanes 1 and 2) or subsequent to secondary DES stimulation (lanes 3–10), as depicted on the top (d, days; h, hours; for details, see “Materials and Methods”). Nuclei from oviducts were isolated and RNA was synthesized in vitro in the presence of [³²P]CTP and then hybridized to filter-bound plasmid or M13 clones (see “Materials and Methods” for a description of probes). Filters were washed and exposed to Kodak X-omat films. The autoradiographs in A, lanes 1 and 2, 3–8, 9, and 10 are from separate experiments. The following quantitative data were obtained from scanning PR-signals (expressed relative to actin). Lane 2: 115% relative to lane 1; lanes 4–8: 40, 60, 90, 60, and 70%, respectively (relative to lane 3); lane 10: 60% relative to lane 9. B, PR gene transcription in DES-treated chicks (lane 1) stimulated for 12 h by progesterone (P, lane 2), dexamethasone (D, lane 3), dihydrotestosterone (T, lane 4), or RU486 (RU, lane 5). The following quantitative data were obtained from scanning PR signals (expressed relative to actin, lane 1 taken as 100%). Lanes 2–5, 120, 100, 100, and 75%.

**Fig. 4.** Effect of DES treatment on the levels of PR mRNA species in the chick oviduct. 10-day-old chicks were treated with DES for 10 days, withdrawn for 4 days, restimulated with DES for 10 days and then withdrawn (W) or maintained on DES (S) for 3 days. Oviduct poly(A)⁺ RNA was isolated, electrophoresed on a 1% agarose-formaldehyde gel (5 [microg] of RNA/slot), transferred onto a nitrocellulose filter and hybridized with [³²P]-labeled PR, ovalbumin, or actin probes (for a description of the probes, see “Materials and Methods”).
to estradiol. In fact, an estradiol-dependent decrease of the promoter activity was observed instead (Fig. 1D, compare lanes 11 and 12). (Note that similar results were obtained with reporter genes containing 4.1-kb cPR 5'-flanking sequences (data not shown).) The chicken estrogen receptor was active in our cotransfection assay, as replacing (−1683/+80)CAT with the vit-tk-CAT reporter gene (Klein-Hitpass et al., 1986) resulted in a strong estradiol-dependent stimulation of CAT activity (data not shown). In summary, neither transient expression studies nor nuclear run-on experiments explain the marked decrease in the amount of cPR mRNA observed on estrogen withdrawal and we, thus, conclude that the estrogen regulation of the cPR level is exerted mainly at a post-transcriptional step.

**DISCUSSION**

**Estrogen Regulation of cPR Expression Occurs at the Post-transcriptional Level**—In chick oviduct, cPR levels are positively regulated by estrogen and withdrawal from estrogen stimulation results in a marked decrease of progesterone receptors (see Introduction for references). By Northern blot analysis, we show here that this change at the protein level correlates with changes in the amount of cPR mRNAs. However, no change in cPR gene transcription could be detected with oviduct nuclei from estrogen-stimulated and withdrawn chicks. No DHSs were observed in liver and erythrocyte nuclei (Fig. 5B, lanes 1–6 and 12–15, respectively) as compared with naked DNA (lanes 16–22) of laying hens, while kidney nuclei exhibited one DHS (III in Fig. 5B, lanes 7–11). In contrast, in oviduct nuclei of laying hens (Fig. 5B, lanes 23–25) or chicks (Fig. 5C) four DHSs were detected in the 5′ region of the PR gene at about −1.6 (I, more apparent on other blots), −0.7 (II), −0.1 (III), and +0.6 (IV) kb relative to the cap site using probes 1–4 (illustrated in Fig. 5A). Weak DHSs detected less than 1 kb from probe 1 (Fig. 5C, left panel) are probably not genuine since they were seen also with naked DNA (Fig. 5B, lanes 16–22). Most importantly, the pattern and intensity of three DHSs (I, III, and IV) remained very similar with nuclei from stimulated or withdrawn chicks, while site II at −0.7 kb is weaker in nuclei from withdrawn chicks (Fig. 5C; note that in some experiments the differences seen for site II were much less pronounced (data not shown); note also that no estrogen-responsive elements were found at or in the vicinity of DHS II, see “Discussion”). No other DHS could be detected up to 8.1 kb upstream of the cap site when using probes 1, 2, or 4 (Fig. 5 and data not shown).

**FIG. 5.** Mapping of DNase I hypersensitive sites in the 5′ region of the PR gene. Nuclei were isolated and digested with increasing amounts (from left to right for each nuclei preparation) of DNase I (see Kaye et al., 1986). The DNA was then isolated, digested with BglII (B and C, left panel) or HindIII (C, right panel) electrophoresed, blotted, and hybridized with probe 1 (B and C, left panel) or 3 (C, right panel). Fragment lengths are in kilobase pairs (kb). Roman numerals indicate the presence of DHSs and correspond to those given in A. A, restriction map of the 5′ region of the PR gene with the black rectangle corresponding to the first exon of the PR gene (Jettsch et al., 1990). Coordinates are given on the top as distance in kb from the cap site (0). Arrows below indicate the positions of genomic fragments used as probes (1–4) and the direction of mapping. Small boxes with Roman numerals correspond to the DHSs localized in the 5′ region of the PR gene. B, tissue specificity of DHSs in cPR gene 5′-flanking region. Nuclei were prepared from laying hen liver (lanes 1–6), kidney (lanes 7–11), erythrocytes (lanes 12–15), or oviduct (lanes 23–25). Naked DNA, treated similarly, is shown for control purposes (lanes 16–22). C, DHSs in hormonally treated chicks. After a secondary stimulation with DES, chicks were withdrawn (W, lanes 6–11; left panel; lanes 5–9, right panel) or maintained on DES (S; lanes 1–5, left panel; lanes 1–4, right panel) for 3 days and oviduct nuclei were isolated and treated as described above.

known to occur upon hormonal induction or repression of transcription in many genes (Burch and Weintraub, 1983; Zaret and Yamamoto, 1984; Becker et al., 1984; Kaye et al., 1986; Turcotte et al., 1987). That estrogen inducibility of cPR is a post-transcriptional mechanism is further supported by...
data obtained with transiently transfected cPR promoter chimeric reporter genes. Although sequences upstream of the cPR cap site constituted an active promoter when transfected into chicken embryo fibroblasts, no induction of promoter activity was observed in the presence of estradiol and coexpressed chicken estrogen receptor, even when using reporters containing up to 4.1 kb of cPR-flanking sequences. Why we observed some decrease in cPR promoter activity is unclear but may be related to some kind of transcriptional interference (Meyer et al., 1989). Note in this respect that no estrogen-responsive elements, as described for the Xenopus or chicken vitellogenin genes (Klein, Hitpass et al., 1986; Martinez et al., 1987; Burch et al., 1988), were found within 2.3 kb upstream of the cPR cap site (nor 4.7 kb downstream of it, see Jeltsch et al., 1990; Gronemeier et al., 1987). Mechanisms destabilizing cPR mRNA in withdrawn chicks (or, alternatively, stabilizing cPR mRNA in estrogen-stimulated chicks) may, therefore, account for the estrogen dependency of PR levels in chick oviduct. We cannot, however, exclude other mechanisms of post-transcriptional regulation, such as differential RNA processing.

Analysis of the cPR Promoter: Identification of cPR Form A-responsive Elements Which May Act in a Cell-specific Fashion—We recently characterized more than 2 kb of the cPR gene 5'-flanking region (Jeltsch et al., 1989). The immediate 5' region is devoid of any obvious TATA or CCAAT-box, but here we demonstrate, by transient transfection studies, that 867 bp of these upstream sequences constitute a cell-specific promoter. In S1 nuclease mapping experiments with promoter-chimeric cPR-globin constructs, a major protected fragment was obtained, corresponding to initiation at the cap site of cPR mRNA (Gronemeyer et al., 1987). Since activity was observed after transfection in CEY, but not in HeLa, MCF-7 or HepG2, cell-specific factors are apparently required for the cPR promoter to function. As for other TATA-less genes, it is unclear which signals actually trigger transcriptional initiation of the cPR gene (for a discussion, see Jeltsch et al., 1990; Dynan, 1986 and references therein).

By use of in vitro footprinting and transient transfection experiments, we have identified functional PREs in the cPR gene promoter (Figs. 1 and 2). Interestingly, the A but not the B form of cPR induced CAT activity from a cPR promoter-CAT chimeric gene. This is strikingly reminiscent of a similar observation for the ovalbumin gene (Tora et al., 1988). Dose-response curves demonstrated that 867 nucleotides are sufficient for an up to 3.5-fold stimulation by cPR form A (but not by cPR form B) of the cPR promoter. Within this region we detected a potential PRE/GRE (−237 to −252; see Fig. 2) which was able to bind to the cPR DBD in vitro. Although we did not investigate whether auxiliary nuclear factors may further stabilize binding of the cPR DBD (or of the entire receptor) to the upstream PRE/GREs, it is unlikely that such factors play a significant role in vivo, since the presence of the upstream PRE/GREs only marginally increased (3.5-5-fold) the response of (−1683/+50)CAT to cPR form A/B5020.

In contrast to the data by transient transfection and in vitro DNA binding studies, no changes of PR gene transcription were observed in vivo in oviducts from progesterin-stimulated chicks as assayed by nuclear run-on techniques. Similarly, exposure of DES-treated chicks to glucocorticoids or androgens whose cognate receptors activate the same responsive element as PR (Ham et al., 1988 and reference therein) did not affect PR gene transcription in oviduct cells in vivo. One possibility is that the coexistence in oviduct cells of cPR form A and B "masks" the action of form A since the inactive form B may compete for the common binding sites and/or form hetero-dimers (Meyer et al., 1990). We note in this respect that the expression of the two cPR isoforms is developmentally regulated in chick ovipud. However, this hypothesis does not explain the induction by progestins of ovalbumin gene transcription in DES-primed chicks. (In transiently transfected CEY the ovalbumin promoter is cPR form A (but not form B)-inducible (Tora et al., 1988).) Therefore, cell- and/or promoter-specific mechanisms may determine the different inducibilities of ovalbumin and PR gene transcription in vivo.

Irrespective of the origin of the differences between transfection and run-on data, the observations that two genes are differentially regulated by forms A and B of cPR (Tora et al., 1988; this study) and that PR mRNAs exist which encode either form (Jeltsch et al., 1990) suggest the possibility that the two forms may be expressed differently. Differential expression in a developmental and/or tissue-specific manner may establish a system to regulate classes of genes by their sensitivity toward stimulation by form A and/or form B and thus provide a (still lacking) rationale for the existence of two forms for the cPR.

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