A Limiting Factor Mediates the Differential Activation of Promoters by the Human Progesterone Receptor Isoforms*

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The two transcription activation functions (TAFs) of the human progesterone receptor (hPR) have been characterized. TAF-1, located in the N-terminal region A/B, has been narrowed down to a 91-amino acid sequence, which is sufficient for transcription activation in chimeras with the GAL4 DNA binding domain. Both hPR TAF-1 and TAF-2 activate a minimal promoter in yeast. No autonomous TAF could be found in the N-terminal 164 amino acids (designated AB3) which are responsible for the differential activation of promoters by the hPR isoforms A and B. Reduction of the target gene promoter complexity did not alter this differential activation, indicating that AB3 does not require additional promoter-bound factors to exert its effect. Instead, the cell specificity of AB3 and its ability to squelch hPR-induced transcription suggest that this differential isoform activity is due to the effect of a limiting factor which binds to region AB3.

The progesterone receptor (PR) is a member of the nuclear receptor family and acts as a ligand-inducible transcription factor. Based on their differential evolutionary conservation, nuclear receptors are commonly divided into six regions, denoted A/B to F, and the domains responsible for DNA (DBD) and hormone binding (HBD) have been identified (Evans, 1988; Green and Chambon, 1988; Beato, 1989). A few DBD residues have been found to be critically involved in the recognition of the cognate hormone responsive elements of the estrogen (ER) and glucocorticoid (GR) receptors (Daniel sen et al., 1989; Mader et al., 1989; Umesono and Evans, 1989), and the HBD of ER has been shown to contain a strong dimerization function (Kumar and Chambon, 1988; Fawell et al., 1990). We and others have demonstrated that ER, PR, and GR contain two transcription activation functions (TAFs), located in the N-terminal region A/B (TAF-1) and in the HBD (TAF-2), respectively (see Fig. 2B; for a review, see Gronemeyer, 1991). TAF-1 and TAF-2 apparently operate in a cell- and promoter-specific fashion, and type I antagonists like RU486 or hydroxy-tamoxifen can act as partial agonists of their cognate receptors, since TAF-1, but not TAF-2, may be active in the presence of these compounds (Berry et al., 1990; Meyer et al., 1990). Evidence has been presented that receptor TAFs exert their action via transcription intermediary factors (TIFs) (Meyer et al., 1989; Tasset et al., 1990), also referred to as coactivators or adapters. While for the GR a 30-amino-acid segment (the so-called tau-2) encompassing the N-terminal border of the HBD was found to activate transcription (Hollenberg and Evans, 1988), no sub-sequence of the ER HBD, which itself activated transcription in the presence of estrogen, was sufficient for transcription activation (Webster et al., 1989). In yeast, the ER activates transcription by virtue of its TAF-1, while TAF-2 was not functional with a reporter gene containing the estrogen response element linked to a minimal promoter (Berry et al., 1990). In contrast, the GR activated transcription in yeast of the iso-l-cytochrome c promoter in a hormone-dependent manner, even when the N-terminal region A/B was deleted (Wright et al., 1990).

The human PR (hPR) exists as two isoforms, termed B and A (Schrader and O'Malley, 1972), which are generated by initiation of translation at two in-frame ATGs (amino acid position 1 and 164, respectively). Two different estrogen-regulated promoters are responsible for the synthesis of isoform A- and isoform B-specific hPR mRNAs (Kastner et al., 1990c), and it has been demonstrated that the two hPR isoforms differently activate transcription from target gene promoters (Tora et al., 1988; Kastner et al., 1990c). The observations that hPR/RU486 induced transcription of some (but not all) target genes whereas mutant receptors lacking the N-terminal region A/B were inactive in the presence of RU486 (Meyer et al., 1990) suggested that target gene activation in the presence of RU486, like the isoform-specificity of activation of transcription in the presence of the agonist, may be linked to specific characteristics of the PR region A/B.

In an attempt to understand how the two hPR isoforms can differentially regulate target gene expression, we have localized the core of TAF-1 in the region A/B and investigated the effect of the region that differs between the two isoforms (amino acids 1 to 164, hereafter referred to as region AB3) on activation of transcription. These studies suggest the existence of limiting factor(s) which interact with the hPR region AB3 and which is required for transcription activation not only by isoform B but also by isoform A. Furthermore, we show that both TAF-1 and TAF-2 of hPR activate transcription from a minimal promoter in yeast.
MATERIALS AND METHODS

Recombinants for Mammalian Cell Studies—The reporter gene 17M-tk-CAT and the expression vectors hPR1, hPR2, hPR3, and hPR5 have been described (Webster et al., 1988a, 1988b; Meyer et al., 1989; Kastner et al., 1990a, 1990b). hPR5 originates from the replacement of the BamHI-XhoI fragment in hPR2 by the corresponding fragment of hPR5, thus translation is initiated, as in hPR2, at the second in-frame ATG (Kastner et al., 1990c). The Gal-hPR(AB) chimeras were constructed using PCRamplification with hPR1 as template. All constructs were verified by sequencing. The sequences of the primers used for the various constructions are available on request. For Gal-hPR (AB3, 4, 5, and 11) primers were used which generated 5'-XhoI, 3'-SacI-flanked DNA sequences encoding the hPR amino acids 1-164, 165-290, 298-397, and 515-546, respectively, and were inserted into the corresponding sites of p4GmPolyl (Webster et al., 1989). Gal-hPR (AB0) was generated by inserting in-frame a 5'-XhoI, 3'-BamHI-flanked PCR fragment encoding amino acids 1-24 into the corresponding sites of Gal-hPR(AB1) (Meyer et al., 1990). Gal-hPR(AB2) was constructed by ligating in a single reaction the XhoI-ApaI fragment (amino acids 165-207) of Gal-hPR(AB4), the Apal-HindIII fragment (amino acids 208-456) of Gal-hPR(AB30) and the HindIII-BamHI fragment (amino acids 457-546) of hPRmut1 into the XhoI-BamHI sites of p4GmPolyl II. In hPRmut1, a kind gift of J. Ji (Laboratoire de Génétique Moléculaire des Eucaryotes, Strasbourg, France), the amino acids 547 to 550 were replaced by site-directed mutagenesis in-frame with the sequence GSTM (5'-GGA TTC ACC ATG), thus introducing a BamHI site. Gal-hPR(AB6) was obtained by inserting an XhoI-StyI fragment (corresponding to amino acids 398-456) and a HindIII-BamHI flanked fragment (corresponding to amino acids 457-546) into the XhoI and BamHI sites of p4GmPolyl II. Gal-hPR(AB7) originates from insertion of the SalI-SacI-flanked fragment (hPR amino acids 296 to 546) of Gal-hPR(AB2) into the corresponding sites of Gal-hPR(AB3). Gal-hPR(AB8) and Gal-hPR(AB12) were obtained by inserting the PCR-generated XhoI-StyI-flanked fragment encompassing amino acids 1-164 into the XhoI site of Gal-hPR(AB7) and Gal-hPR(AB6), respectively. Gal-hPR(AB9) and Gal-hPR(AB10) express the hPR sequences from amino acids 458 to 468 and 526 to 536, respectively, due to an in-frame insertion of the corresponding SalI-SacI-flanked PCR-generated fragments into the XhoI-SacI sites of p4GmPolyl II. To construct Gal-hPR(AB13), the SalI fragment of hPR0 was blunt-ended with Klenow polymerase and cloned into the similarly blunt-ended BamHI site of p4GmPolyl II, resulting in an in-frame fusion protein. Gal-hPR(tau2) originates from a PCR-generated fragment (5'-XhoI, 3'-SacI) encompassing the sequence coding for hPR amino acids 691 to 730 which was inserted into the corresponding sites of p4GmPolyl II.

Recombinants for Yeast Cell Studies—yPR1, yPR2, yPR3, and yPR5 originate from pY0E1 (Metzger et al., 1988) by replacing the XmaI-XhoI fragment containing the ERE sequence with a Notl site-containing oligonucleotide. A palindromic PRB sequence (as described (Eul et al., 1989)) was inserted into this unique Notl site, generating pY0MPRE. To construct yPR1, the BglII-BglII fragment of hPR0/24-2 was inserted into the BglII site of yPR(0)RE, downstream of the PGK promoter. hPR0/24-2 was created by site-directed mutagenesis, introducing BglII sites after position 711 and 3612, and a yeast sequence for translation initiation (710-AGATCTGGGAGTCCAAAAAAAATGA). The insertion of the BamHI fragment of hPR0/24-2 into the BamHI site of yPR(0)RE created yPR5. To generate yPR5, the BglII-XhoI fragment of hPR0/24-2 was replaced by the corresponding one of hPR5 (Meyer et al., 1990), and the BglII fragment of the resulting construct was transferred into yPR(0)RE.

Transcription Activation in Yeast—Transfections and β-galactosidase assays were carried out as described by Metzger et al. (1988).

Transient Transfection and Cat Assays in Mammalian Cells—Transfections were done by the standard calcium phosphate coprecipitation technique. If not stated otherwise, HeLa or CV:1 cells were cotransfected with 2 μg of the various expression vectors, 2 μg of the reporter gene, and 1 μg of the internal control transfection (Bocquel et al., 1992a; and CH10 Pharmacia LKB Biotechnology Inc.). Cat assays with normalized extracts were performed as described (Grommeyer et al., 1987; Bocquel et al., 1989).

Immunoblot—Western blots, performed with all chimeric constructs, confirmed similar expression levels, and were done according to standard procedures, using a mix of two monoclonal antibodies (2G3V and 3GV2) directed against the GAL4 DNA binding domain.2 The secondary rabbit anti-mouse antibody was used at a dilution of 1 to 400.

RESULTS

Differential Activation of a Minimal Promoter by the hPR Isoforms—Previous experiments demonstrating a different activity of the human (hPR) or chicken PR isoforms have been performed with complex promoters (Tora et al., 1988; Kastner et al., 1990c). To investigate whether this isoform specificity is dependent on the presence of additional factors binding to the reporter gene promoter, we tested the activation of minimal promoters (GRE-TATA, GREx2-TATA), which contain only a progestin/glucocorticoid response element and the thymidine kinase TATA box (Schmid et al., 1989). Interestingly, both reporters are differentially activated by the hPR isoforms; hPR form B (expressed from hPR1) is about five times more active than form A (expressed from hPR2) (Fig. 1B, compare lanes 3 and 6, and lanes 11 and 12; Fig. 1C). Similar differences were observed with the truncated receptors hPR5 and hPR52 (illustrated in Fig. 1A), which correspond to form B and A, respectively, in which the hormone binding domain has been deleted (Fig. 1B, compare lanes 1 and 2, and lanes 9 and 10). (Note that hPR3, which corresponds to a receptor lacking the N-terminal region A/B (Fig. 1A), displayed a strong homosynergistic effect (for a definition see “Discussion” and Tasset et al. (1990)), since it activated strongly GREx2-TATA-CAT (Fig. 1B, lane 15), while hardly any activation was seen with the monomeric GRE-TATA-CAT (lane 7).) We conclude that the binding of additional factors to a complex promoter is not required for the isoform specificity of transcription activation.

TAF-1 of hPR Is Contained within a Sequence of 91 Amino Acids and Its Activity Is Modified by Region AB3—In order to understand how the isoforms A and B of hPR could differentially activate target genes it is essential to know whether a TAF is present in region AB3 (note that a TAF is an amino acid sequence which, when attached to a heterologous DNA binding domain, is sufficient to stimulate transcription from cognate reporter genes). Different portions of region A/B were linked to the DNA binding domain of the yeast transcription activator GAL4, giving rise to the chimeras Gal-hPR(AB0 to AB6) (illustrated in Fig. 2B). Vectors expressing these chimeras were cotransfected with the cognate reporter recombinant 17M-tk-CAT (Webster et al., 1988a, 1988b) (Fig. 2A) into HeLa cells, and transcription activation was determined by CAT assay. Gal-hPR(AB0) containing region A/B of isoform B (Fig. 2B) activated transcription nearly twice as much as Gal-hPR(AB2) which contains the corresponding region of hPR isoform A (Fig. 3A, lanes 4-7). Therefore, these two chimeras mimic the differential transcription activation seen with the full-length isoforms and the MMTV-CAT or GRE-TATA-CAT reporter genes, providing further evidence that only the regions A/B but not, for example, differential DNA binding of the two PR isoforms to a particular template, is responsible for the isoform specificity of transcription activation.

2 J. White, C. Brou, Y. Lutz, V. Moncolin, and P. Chambon, submitted for publication.
The two hPR isoforms differently activate a minimal promoter. A, schematic illustration of hPR1 and hPR2, expressing isoforms B and A, respectively, which originate from different messengers by translational initiation at ATG1 or ATG2 (for a review see Gronemeyer et al., 1991). Open boxes refer to the DNA (region C) and hormone binding domains (region E). The hPR regions expressed in the truncation mutants hPR3, hPR5, and hPR52 are illustrated below. B, HeLa cells were transiently transfected with hPR1 (expressing hPR form A; lanes 3, 4, 11, 12) or hPR2 (expressing form A; lanes 5, 6, 13, 14) and a reporter gene containing a minimal promoter with one (GRE-TATA-CAT; lanes 1–8) or two (GREX2-TATA-CAT; lanes 9–16) PRE/GREs and cultured in the presence or absence of 20 nM R5020 as illustrated. Similar transfections were done with hPR5 (containing the A/B region of isoform B and lacking the HBD; lanes 1 and 9) and hPR52 (containing the A/B region of form A and lacking the HBD; lanes 2 and 10), and with hPR3 (lacking the region A/B; lanes 15 and 16). In all cases 1 µg of the corresponding expression vectors was transfected. C, quantitation of the CAT assay. Data are expressed relative to 1 unit of β-galactosidase produced from the internal control recombinant pCH110. Note the strong homosynergistic effects of hPR3, hPR5, and hPR52 on the reporter with a tandem response element.

Apparently, the region between amino acids 398 and 455 is dispensable for transcription activation, since Gal-hPR(AB13) (Fig. 2B) was as active as Gal-hPR(AB6) (Fig. 3A, lane 14). Note that all chimeras have been expressed in HeLa (and Cos) cells with similar efficiencies (data not shown). Thus, we conclude that (i) AB13 contains the only TAF present in hPR region A/B, and (ii) the differential activation by the two isoforms is not due to the presence of a separate autonomous TAF within region AB3, but rather may be due to the modulatory effect of this sequence on TAF-1.

Further dissection of the amino acids stretch from 456 to 546 showed that none of the subsequences tested contained an autonomous TAF. Even though the sequences expressed in Gal-hPR(AB9), (AB10), or (AB11) (illustrated in Fig. 2B) are to some degree conserved in the chicken, mouse, rabbit, and human homologues (see Fig. 6), none of these regions alone constituted a TAF (see Fig. 3A, lanes 10–12). We conclude that the minimal region tested in this study that is sufficient for transcription activation, and therefore corresponds to TAF-1, is the 91-amino acid stretch present in Gal-hPR(AB13).

Interestingly, although the region AB3 does not contain a TAF, it can modify the activity of TAF-1 when present in the same protein. Both Gal-hPR(AB12) and Gal-hPR(AB8) are more potent activators than the corresponding ones lacking region AB3 Gal-hPR(AB6) and Gal-hPR(AB7); Fig. 3A, compare lanes 13 and 7, and lanes 9 and 8, respectively; Fig. 3C). In summary, isoform specificity of transcription activation is, at least in part, due to a modifying effect of region AB3 on TAF-1 of hPR.

Cell Specificity of the Modulatory Function Present in Region AB3—We previously observed a different cell specificity of the chicken PR TAF-1 and TAF-2 (Bocquel et al., 1989), most likely due to a different activity and/or abundance of the corresponding transcription intermediary factors (Meyer et al., 1989) in the corresponding cell lines. Therefore, we investigated whether the effect of region AB3, which possibly may be mediated by such factors, is different in the two cell lines. Indeed, in CV1 cells, Gal-hPR(AB2) was at least as efficient as Gal-hPR(AB0) in activation of the 17M-tk-CAT reporter gene (Fig. 3B, lanes 2 and 3; see Fig. 3C for the quantitation of transcription activation in HeLa and CV1 cells). Note that also in CV1 cells AB13 was the minimal region containing an autonomous TAF (Figs. 3B and C). The different activity of Gal-hPR(AB0) and Gal-hPR(AB2) in HeLa and CV1 cells was not due to a differential expression or degradation, since immunoblots demonstrated that the relative levels of the two proteins were the same in both cell types (Fig. 4, lanes 2–5). These results suggest that the effect...
the top were transfected together with the 17M-tk-CAT as in with the 17M-tk-CAT reporter (Fig. 2A) (indicated at the top) were cotransfected with the 17M-tk-CAT as in with the 17M-tk-CAT reporter (Fig. 2A) (indicated at the top) were cotransfected

of the GAL4 reporter gene transcription, this binding cannot be sufficient for transcription activation. The factor may increase TAF-1 efficiency by interacting with the cognate TIF(s). If this were true, it should be possible to sequester the TAF-1 TIF(s) by coexpressing high amounts of nuclear AB3 with the consequence that the transcription induced not only by hPR1, but also by hPR2 (which lacks AB3 but expresses TAF-1), is inhibited. In fact, Gal-hPR(AB3) severely inhibited the transcription activation of PRE-tk-CAT by the hPR form B (expressed from hPR1; Fig. 5, compare lanes 1 and 3) and hPR form A (expressed from hPR2; Fig. 5, compare lanes 5 and 7; note that no significant inhibition was seen when Gal-hPR(AB3) was replaced by a vector expressing only the GAL4 DBD (data not shown)). This AB3-dependent inhibition is similar to that seen by transcriptional interference (Meyer et al., 1989) of Gal-ER(EF) in the presence of estradiol (Fig. 5, compare lanes 1 and 4, and lanes 5 and 8). Interestingly, Gal-hPR(AB3) was not able to inhibit the estrogen-induced transcription of an ERE-tk-CAT reporter gene (data not shown), suggesting that the AB3 factor cannot sequester TIFs corresponding to the ER TAFs.

RU486 Is a Partial Agonist for hPR in Yeast—In yeast transcription activation of a minimal promoter by the human estrogen receptor occurs mainly by virtue of its TAF-1 (Metzger et al., 1988; Tora et al., 1989; Berry et al., 1990). We have used the same type of reporter gene construction and replaced the hER sequences with those of the hPR in order to monitor the activity of the hPR TAFs in yeast. hPR activated the β-galactosidase reporter gene transcription in a ligand-dependent fashion, to similar levels as hER (Table I). Some transcription activation was seen also in the presence of RU486,

![Fig. 3. A, the amino acid stretch 456 to 546 contains the only TAF present in region A/B of hPR. B, the chimeras depicted in Fig. 2B (indicated at the top) were cotransfected with the 17M-tk-CAT reporter (Fig. 2A) into HeLa cells, and CAT assays were performed according to standard procedures. Note that chimeras expressing region AB3 (Gal-hPR(AB8) and Gal-hPR(AB12)) are more active than the corresponding chimeras lacking AB3 (Gal-hPR(AB7) and Gal-hPR(AB6)), although region AB3 does not contain an autonomous TAF. All chimeras were expressed at similar levels (Fig. 4 and data not shown). B, the stimulatory effect of region AB3 is not observed in CV1 cells. The chimeras depicted at the top were transfected together with the 17M-tk-CAT as in A but into CV1 cells. C, quantitation of the results shown in A and B. Similar results were obtained in at least four independent transfections. In all cases, when transfections were done in HeLa cells, Gal-hPR(AB0) was more active than Gal-hPR(AB2), while it was equal or less active than Gal-hPR(AB2) when CV1 cells were transfected. The data were quantified relative to 1 unit of β-galactosidase originating from the cotransfected internal control recombinant pCH110 (Broquet et al., 1989).]

![Fig. 4. Gal-hPR(AB0) and Gal-hPR(AB2) are expressed at similar relative levels in HeLa and CV1 cells. HeLa (lanes 1–3) and CV1 cells (lanes 4–6) were transfected with 5 μg of either Gal-hPR(AB0) (lanes 3 and 4) or Gal-hPR(AB2) (lanes 5 and 6), and immunoblots were prepared of whole-cell extracts, using a monoclonal antibody which recognizes the GAL4 DBD (see "Materials and Methods"). In lanes 1 and 6, the "empty" expression vector was transfected instead of the chimeras.]

![Progestin-Responsive (PRE-TATA) promoter (for details see "Materials and Methods"). For comparison, basal and induced levels for the (Anti)progestin-dependent transcription were cotransfected with 100 ng hPR1 (expressing isoform B; lanes 1–4) or hPR2 (expressing form A; lanes 5–8) in the absence (lanes 1 and 5) or presence of 3 μg Gal-hPR(AB3) (lanes 3 and 7). As a positive control for transcriptional interference (Meyer et al., 1989), hPR1 and hPR2 were cotransfected with Gal-ER(EF) expressing the HBD and TAF-2 of the human estrogen receptor (lanes 4 and 8). Hormones (E2, estradiol; R5020) were administered as indicated.

### Table I

|          | Basal | R5020 | RU486 | E2  |
|----------|-------|-------|-------|-----|
| hPR1     | 20    | 1100  | 385   |     |
| hPR3     | 10    | 500   | 30    |     |
| hPR5     | 165   | 165   | 165   |     |
| HEG0     | 20    | 1200  |       |     |

Evidence for the Existence of a Factor(s) Which Is Involved in the Modulatory Function of AB3—The above observed cell specificity could be mediated by a factor which binds to the region AB3. However, since Gal-PR(AB3) did not stimulate the GAL4 reporter gene transcription, this binding cannot be
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**Fig. 6. Alignment of hPR TAF-1 contained in Gal-hPR(AB13) with the corresponding sequences of rabbit (rPBR), mouse (mPBR), and chicken (cPBR) progesterone receptors. Asterisks below the sequences indicate amino acid identity; points indicate similarity. Gaps have been introduced into the mPBR and cPBR sequence for optimal alignment. AB regions present in chimeras expressing a subsequence of AB13 are depicted.**

The progesterone receptor is the minimal segment of region A/B

1. **Dobson corresponding to region AB13, severely affected the ability of the full-length cPR form Tjian, 1991), C/EBP (Pei and Shih, 1991), CTF/NF1 (Mermod et al., 1989), and SNF5 (Laurent et al., 1990). TAF-1 is not particularly rich in acidic amino acids, and no clear secondary structure prediction was possible (program PCGENE). The only characteristics of TAF-1 are its hydrophobicity and the finding that it is flanked by two regions which are predicted to have the highest flexibility in this area (N-terminal: 451-457 and 468-474; C-terminal: 550-556). Although we have obtained compelling evidence that the PR TAF-1 interacts with a TIF, which is distinct from the general transcription factors (Shemshedini et al., 1992), we do not know whether the cognate TIF of TAF-1 is specific for the PR or recognizes a binding interface common to “proline-rich” activation domains.

**Involvement of a TIF in the Isoform Specificity of Transcription Activation—**Nuclear receptor isoforms, initially a peculiarity of the progesterone receptor (Schrader and O’Malley, 1972), are known to exist also for the retinoic acid (Kastner et al., 1990b; Leroy et al., 1991; Zelent et al., 1991) and thyroid hormone (Izumo and Mahdavi, 1988; Mitsuhashi et al., 1988; Yoaita et al., 1990) receptors. hPR isoforms originate by alternative promoter usage, while RAR isoforms are generated also by alternative splicing. For the chicken and human PR, we demonstrated the functional difference between the two isoforms A and B, since some promoters responded differently to the two proteins (Torra et al., 1988; Kastner et al., 1990c). The hPBR isoforms differ only in an additional 164 amino acids (region AB3) added N-terminally of form A to give form B. Thus, at least four mechanisms could account for the isoform/promoter specificity: (i) region AB3 and the A/B region of form A contain two separate TAFs with different promoter-specificities, (ii) region AB3 affects DNA binding of form B positively or negatively, depending on the template (e.g. by steric hindrance), (iii) AB3 modulates the activity of TAF-1 or TAF-2 at the transcriptional level, e.g. by binding to a transcription modulatory factor which augments TAF activity and may, in addition, differently synergize with the promoter environment of the target gene.

In this study, we have reduced the complexity of both target gene and activator and asked whether we are able to observe isoform/target gene-specific activation. Indeed, the use of minimal promoters revealed that, like with the MMTV-CAT (Kastner et al., 1990c), form A (hPR2) activated transcription less than form B (hPR1). Moreover, in HeLa cells this differential activity could be reproduced with the regions ABC (hPR5 and hPR52) of the two isoforms, as well as with the corresponding GAL4 chimeras, indicating that the positive effect of AB3 occurs independent of the promoter and the activator DBD; and that TAF-2 plays, if at all, a minimal role. In addition, hPR52 richly significantly stimulates MMTV-CAT transcription, while weak activation was observed with hPR5 (data not shown). All these data indicate that hPR region AB3 can positively influences hPR-induced transcription both of minimal and complex promoters. However, the Gal-hPR(AB3) was unable to stimulate transcription of the cognate reporter gene, demonstrating that region AB3 does not contain an autonomous TAF. Interestingly, differential activity of Gal-hPR(AB0) and Gal-hPR(AB2) was observed in HeLa but not in CV1 cells, suggesting that a cell-derived factor may contribute to the effect of region AB3. Therefore, we investigated whether a modulatory factor might bind to this sequence and further stimulate hPR TAF activity. We assumed that this factor may be expressed in limiting amounts and tested this hypothesis by squelching/transcriptional interference experiments (Gil and Ptashne, 1988; Meyer et al., 1989). In fact, coexpression of Gal-hPR(AB3) reduced hPR form B/R5020-induced transcription. Thus, we conclude that AB3 interacts with a component which is required for transcription activation of hPR. The AB3 cognate factor may be specific for PR, since Gal-hPR(AB3) was unable

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to squelch/interfere hER/estrogen-induced transcription. Interestingly, Gal-hPR(AB3) could also squelch hPR2-induced transcription, suggesting that the AB3 factor has some affinity for the hPR TIP(s) which are then (indirectly) sequestered by AB3. Also, the AB3 factor may be involved in transcription activation even in the absence of region AB3.

In the case of the ovalbumin promoter, AB3 apparently exerts a negative effect, since this promoter is preferentially activated by hPR form A (Kastner et al., 1990c). We do not know whether this effect is also mediated by the AB3 cognate factor or whether any other of the above described mechanisms is involved. The analysis of this phenomenon is complicated by the fact that the ovalbumin promoter is not active in heterologous cells.

The TAFs of the Estrogen, Progesterone, and Glucocorticoid Receptors Are Different—ER TAF-2 homosynergizes very strongly, while TAF-1 does not (the term "homosynergy" describes the synergistic activation of transcription by a given TAF when activating a promoter providing more than one cognate response element; Tora et al., 1989; Tasset et al., 1990). In contrast, in the PR, also TAF-1 homosynergizes (synergy factor 4.1 (hPR5) and 3.4 (hPR52); the synergy factor is the ratio between the stimulation of transcription seen when two activator molecules can bind to the promoter over twice the stimulation seen when only one activator can bind to the same promoter), though not as strongly as TAF-2 (no synergy factor can be given, since no activation of the monomeric GRE-TATA-CAT was measured in the presence of hPR3 and hormone), and contributed to the overall synergy factor of 16.4 for hPR form B and 11 for form A (compare conversion rates given for Fig. 1). We conclude therefore that PR and ER TAF-1 belong to different classes of activators. Note in this respect that no sequences homologous to hPR AB13 could be found in ER region AB.

Apparently, also the TAF-2 of ER and PR are substantially different from each other, as is revealed by comparing their ability to activate minimal promoters in yeast. No stimulation was observed with activators containing ER TAF-2 as the only activation domain (Berry et al., 1990), while hPR3 (containing only hPR TAF-2) strongly activated transcription from the same promoter (containing a PRE at the place of the ERE), as did hPR5 (expressing TAF-1).

Thus the two TAFs of ER and PR are likely to belong to functionally different classes of transactivators, and it is tempting to speculate that their cognate TIP(s) are different. This does, of course, not exclude squelching between these activators, since the different TIPs of ER and PR may interact with one common intermediary factor, as discussed by Tasset et al. (1990).

PR TAF-2, but Not Necessarily TAF-1, Is Inactive in the Presence of RU486—We reported previously that PR TAF-2 is inactive in the presence of the antiprogestin RU486, while TAF-1 could be active when choosing PRE-tk-CAT as reporter gene. Very similar results were obtained in yeast cells (Table I), indicating that RU486 may have the potential of a TAF-1-specific agonist. However, it has to be pointed out that RU486 binding may affect TAF-1 activity, since hPR form A does not activate PRE-tk-CAT in the presence of RU486, although hPR52 (corresponding to a form A lacking the region E) activates the same promoter (data not shown). Thus, it appears that also TAF-1 can be affected by RU486 binding to the PR, perhaps depending on the promoter architecture.

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