To provide a more direct test of whether phosphorylation exerts a causal effect on the protein kinase activity, metaphase extracts containing active MPF were prepared from unfertilized eggs and incubated with [$\gamma$-$^{32}$P]ATP. We have previously shown that addition of calcium to such preparations inactivates MPF and converts chromosomes into interphase nuclei[22]. As shown in Fig. 4, *in vitro* inactivation of MPF led to a marked decline in H1 kinase activity, and p34$^{cdc2}$ became heavily phosphorylated. In attempts to perform the converse experiment, we have so far been unable to dephosphorylate interphase p34$^{cdc2}$ with protein phosphatases 1 or 2A, or acid or alkaline phosphatase. Possibly only p34$^{cdc2}$ complexed with other proteins at the G2/M border is susceptible to dephosphorylation. The direct phosphorylation and inactivation of p34$^{cdc2}$ *in vitro* (Fig. 4), however, provides strong evidence for a causal effect of phosphorylation on p34$^{cdc2}$. The apparent inactivation of p34$^{cdc2}$ by phosphorylation is only the second example of direct inactivation of a serine/threonine kinase by another kinase, the classic case being phosphorylation and inactivation of myosin light-chain kinase by cAMP-dependent protein kinase[23].

It has long been known from genetic studies in *S. pombe* that expression of the *cdc2*$^+$ gene is essential for the G2 → M transition[5]. Its activity appears to be highly regulated because of the large number of other *cdc* genes that control its function. Notable among these other elements are the products of the *wee1*$^+$ gene, whose expression inhibits *cdc2*$^+$ function, and the *nim1*$^+$ gene, whose product activates *cdc2*$^+$ by a mechanism that involves *wee1*$^+$ (refs. 24, 25). Both *nim1*$^+$ and *wee1*$^+$ protein products contain consensus sequences for protein kinases. The possibility suggested by these genetic studies, that *wee1* might directly inactivate p34$^{cdc2}$ by phosphorylation, is consistent with our biochemical finding that inactivation of p34$^{cdc2}$ kinase activity in the cell cycle is correlated with an increase in its $^{32}$P content (Figs. 2-4). But, further work is needed to identify directly the protein kinase(s) that phosphorylate p34$^{cdc2}$ during the cell cycle.

The oscillations in H1 kinase activity presented here follow closely the reported oscillations in MPF activity during oocyte maturation and after egg activation[20,21]. At present we do not know if the changes in phosphorylation of p34$^{cdc2}$ and its kinase activity are sufficient to regulate MPF activity or whether additional changes in the association or activity of the 45K subunit of MPF are also required. Because p45 is a substrate for p34$^{cdc2}$ in the MPF complex[9], it is possible p45 phosphorylation would decrease upon p34$^{cdc2}$ inactivation and be important for loss of MPF activity. In any case it seems likely that phosphorylation and inactivation of p34$^{cdc2}$ contribute to regulation of MPF activity during the cell cycle. The convergence of evidence for this from both the biochemical and genetic point of view suggests further work on this regulation in the *Xenopus* egg cell-free system will lead to isolation of the kinase(s) that regular p34$^{cdc2}$. □

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1. Simanis, V. & Nurse, P. *Cell* **45**, 261-268 (1986).
2. Lee, M. G. & Nurse, P. *Nature* **327**, 31-35 (1987).
3. Reed, S. I., Hadinger, J. A. & Lorincz, A. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4055 (1987).
4. Draetta, G., Brizuela, L., Potashkin, J. & Beach, D. *Cell* **50**, 319-325 (1987).
5. Lee, M. G. & Nurse, P. *Trends Genet.* 287-290 (1988).
6. Gautier, J., Norbury, C., Lohka, M., Nurse, P. & Maller, J. *Cell* **54**, 433-439 (1988).
7. Labbe, J.-C., Lee, M. G., Nurse, P., Picard, A. & Doree, M. *Nature* **325**, 251-254 (1988).
8. Dunphy, W. G., Brizuela, L., Beach, D. & Newport, J. *Cell* **54**, 423-431 (1981).
9. Lohka, M., Hayes, M. K. & Maller, J. L. *Proc. natn. Acad. Sci. U.S.A.* **85**, 3009-3013 (1988).
10. Tachibana, K., Yanagashima, N. & Kishimoto, T. *J. Cell. Sci.* **88**, 273-281 (1987).
11. Kishimoto, T., Kuriyama, R., Kondo, H. & Kanatani, H. *Expl. Cell Res.* **137**, 121-126 (1982).
12. Wittenberg, C. & Reed, S. I. *Cell* **54**, 1061-1072 (1988).
13. Masaracchia, R.A., Maller, J. L. & Walsh, D. A. *Arch. Biochem. Biophys.* **194**, 1-12 (1979).
14. Cicirelli, M. F., Pelech, S. L. & Krebs, E. G. *J. biol. Chem.* **263**, 2009-2019 (1988).
15. Labbe, J.-C., Picard, A., Karsenti, E. & Doree, M. *Devl Biol.* **127**, 157-169 (1988).
16. Arion, D., Meyer, L., Brizuela, L. & Beach, D. *Cell* **55**, 371-378 (1988).
17. Maller, J. L., Wu, M. & Gerhart, J. C. *Devl Biol.* **58**, 295-312 (1977).
18. Doree, M., Peaucellier, G. & Picard, A. *Devl Biol.* **99**, 489-501 (1983).
19. Draetta, G. & Beach, D. *Cell* **54**, 17-26 (1988).
20. Wasserman, W. J. & Smith, L. D. *J. Cell Biol.* R15-R22 (1978).
21. Gerhart, J. C., Wu, M. & Kirschner, M. W. *J. Cell Biol.* **98**, 1247-1255 (1984).
22. Lohka, M. J. & Maller, J. L. *J. Cell Biol.* **101**, 518-523 (1985).
23. Conti, M. A. & Adelstein, R. S. *J. biol. Chem.* **256**, 3178-3181 (1981).
24. Russell, P. & Nurse, P. *Cell* **49**, 559-567 (1987).
25. Russell, P. & Nurse, P. *Cell* **49**, 569-576 (1987).

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# Glucocorticoid- and progesterone-specific effects are determined by differential expression of the respective hormone receptors

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**ALTHOUGH glucocorticoids and progestins control vastly different physiological processes, the receptors mediating the effects of these hormones interact with the same DNA sequences[1-4]. Transfer experiments involving synthetic genes[5] and *in vitro* binding studies[6] have shown that progesterone and glucocorticoid receptors both recognize the same 15-base pair DNA element (TGTACAGGATGTTCT), raising the question of how the two steroids affect gene expression selectively. We considered the possibility that their selectivity arises from either the differential expression of the receptors in target cells or the differential dependence of receptor function on additional transcription factors. To test these alternatives we introduced a progesterone-receptor expression plasmid into the rat hepatoma cell line Fto2B-3 which contains glucocorticoid receptor but is devoid of progesterone receptor. We report that expression of the progesterone receptor in Fto2B-3 cells renders endogenous glucocorticoid-regulated genes inducible by progestins. Our data show that the responsiveness of a cell to external stimuli can be reprogrammed by the expression of a single transcription factor and that differential expression of hormone receptors is at least one mechanism by which steroid-specific gene activation is achieved.**

To investigate whether glucocorticoid-regulated genes in the liver, a major target for glucocorticoids which does not express progesterone receptor[7], could be made progesterone-responsive we stably introduced an expression plasmid coding for the chicken progesterone receptor[8] (Fig. 1*a*) into Fto2B-3 rat hepatoma cells[9,10]. In two of the stably transformed clones (clone 1, clone 2) the levels of progesterone receptor were measured by steroid-binding assays[11] (Fig. 1*b*). To assay for functional progesterone receptor we analysed induction mediated by the 15-base pair (bp) progestin/glucocorticoid response element (GRE) upstream of the thymidine kinase (*Tk*) promoter[5] in transient transfections of clone 1 and clone 2 cells (Fig. 1*c*). Administration of either the synthetic progestin R5020 or the synthetic glucocorticoid dexamethasone resulted in similar inductions of chloramphenicol acetyltransferase (CAT) expression from the plasmid pGRE15A (ref. 5) in clone 1 and clone 2 cells. R5020 did not induce CAT expression in the parental cell line Fto2B-3, showing that the induction of *Tk*CAT expression by R5020 is due to specific activation of the chicken progesterone receptor in clone 1 and clone 2 cells.

To test whether expression of the progesterone receptor in clone 1 and clone 2 cells would also allow an endogenous, glucocorticoid-regulated gene to be induced by progestins,

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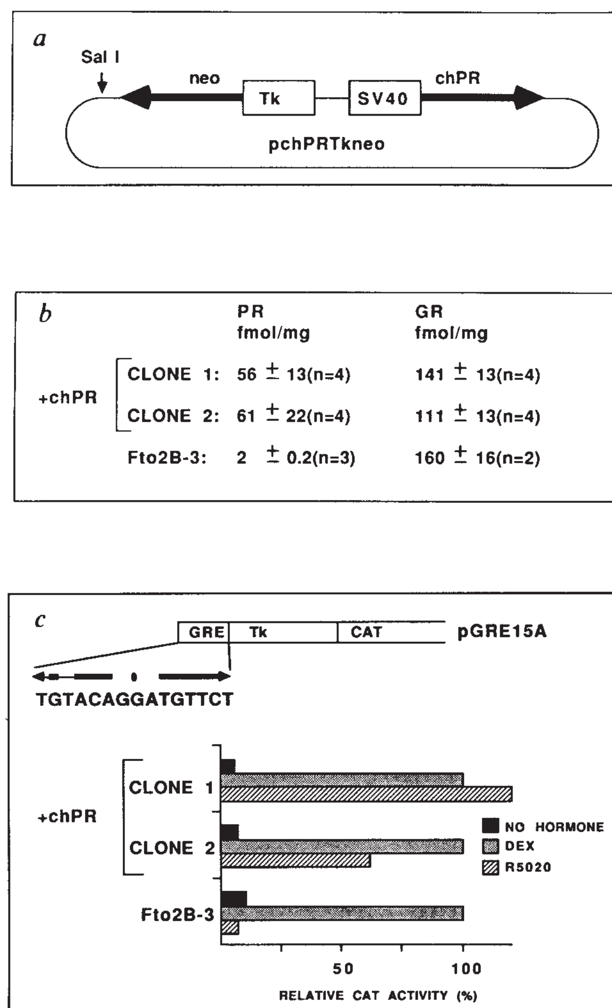
the effects exerted by R5020 on expression of the tyrosine aminotransferase gene[12] (TAT, Fig. 2*a*), the carbamoylphosphate synthetase I (CPS) gene and the argininosuccinate synthetase (ASS) gene (Fig. 2*b*) were analysed. The messenger RNA levels for all three genes were elevated in response to R5020 in transformants expressing chicken progesterone receptor but not in the parental Fto2B-3 cells. Glucocorticoid-regulated genes were, thus, rendered equally responsive to progestins simply by the presence of the progesterone receptor. Hence, receptor content is decisive for steroid selective activation of these genes.




b

| | | PR fmol/mg | GR fmol/mg |
|---|---|---|---|
| +chPR | CLONE 1: | 56 ± 13(n=4) | 141 ± 13(n=4) |
| | CLONE 2: | 61 ± 22(n=4) | 111 ± 13(n=4) |
| | Fto2B-3: | 2 ± 0.2(n=3) | 160 ± 16(n=2) |

FIG. 1 Transformation of rat hepatoma cells with the chicken progesterone receptor. *a*, Map of the chicken progesterone-receptor expression plasmid pchPR*Tkneo*, which contains the cDNA of the B-form of the chicken progesterone receptor (chPR) linked to the SV40 early promoter/enhancer region[8] and the neomycin resistance gene (*neo*) under control of the thymidine kinase promoter of Herpes simplex virus (*Tk*). *b*, Determination of glucocorticoid receptor (GR) and progesterone receptor (PR) levels in cells of clone 1 and clone 2 (Fto2B-3 cells stably transfected with the plasmid pchPR*Tkneo*) and the parental Fto2B-3 cells. Results are expressed as fmol of receptor per milligram of extract protein. Standard deviations were calculated from the results of several determinations ($n$ = number of measurements). The low level of specific binding of R5020 in Fto2B-3 extracts is most likely a consequence of cross-reaction of R5020 with the glucocorticoid receptor[19]. *c*, CAT expression from plasmid pGRE15A is induced equally by R5020 and dexamethasone in cells of clone 1 and clone 2. The plasmid pGRE15A (ref. 5) contains a 15-bp progestin/glucocorticoid response element (GRE) upstream of the thymidine kinase gene promoter (*Tk*) of Herpes simplex virus which drives the expression of CAT. The structure of the plasmid pGRE15A and the sequence of the GRE are shown. pGRE15A was transiently transfected by electroporation. Clone 1, clone 2 and Fto2B-3 cells were induced by either 10 nM R5020, 100 nM dexamethasone (DEX) or no hormone. The results are expressed relative to the glucocorticoid-induced CAT activity which is set to 100%.

METHODS. Details of the construction of pchPR*Tkneo* are available on request. Receptor concentration was measured by the charcoal assay[11]. Clone 1 and clone 2 cells were kept in medium containing 800 μg/ml$^{-1}$ G418 and grown as previously described[9]. To generate transformants expressing the chicken progesterone receptor, Fto2B-3 cells were transfected with pchPR*Tkneo* linearized at the *Sal*I site (see Fig. 1*a*) by either electroporation[9] or by calcium phosphate coprecipitation[20]. Single colonies were screened for progesterone receptor expression by analysing the effect of R5020 and dexamethasone on the expression of transiently transfected pMMTVCAT (ref. 21). Transient transfections, hormone treatment and determination of CAT enzymatic activity were performed as previously described[9].

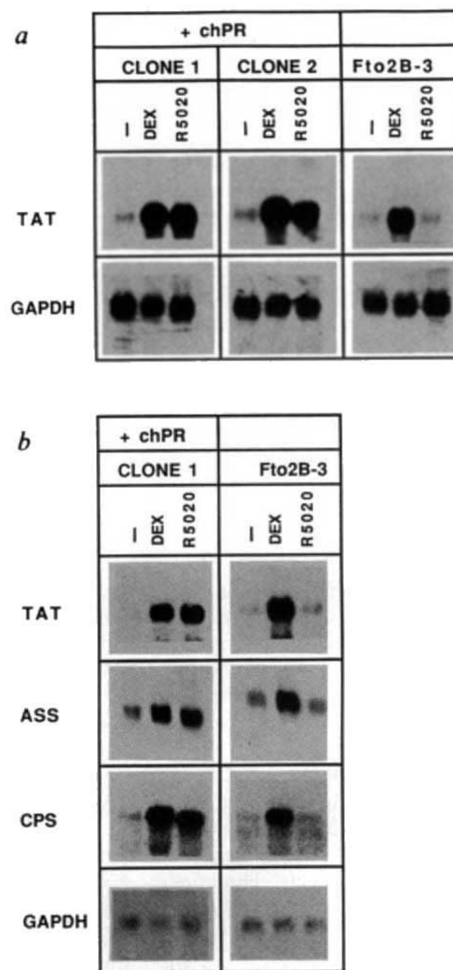



FIG. 2 Expression of the chicken progesterone receptor renders endogenous glucocorticoid-regulated genes inducible by the synthetic progestin R5020. *a*, Northern-blot analysis of tyrosine aminotransferase (TAT) mRNA in clone 1, clone 2 and Fto2B-3 cells following administration of dexamethasone (DEX) or R5020. *b*, Argininosuccinate synthetase (ASS) and carbamoylphosphate synthetase I (CPS) mRNA levels following R5020 or dexamethasone administration. Cells were treated with either no hormone (–), or 10 nM R5020 or 100 nM dexamethasone (DEX). Total RNA was isolated and analysed by the northern-blotting procedure using antisense cDNA probes specific for the various mRNAs. As a control (*a* and *b*) the same filters were rehybridized with a probe specific for glyceraldehyde 3-phosphate dehydrogenase mRNA (GAPDH).

METHODS. Cells were treated for 12 h with either 100 nM dexamethasone, 10 nM R5020 or 0.1% ethanol alone. Total RNA was isolated as previously described[22]. Aliquots (5 μg) were electrophoresed through horizontal, 1% agarose-formaldehyde gels, followed by transfer in 10 × SSC onto a Genescreen membrane (NEN), baking and UV-crosslinking[23]. Hybridizations were performed as previously described[24]. As probes, antisense cDNA transcripts[25] of rat ASS, rat CPS (both provided by S. Morris), rat GAPDH[26] and rat TAT[27] were used. Autoradiographs were exposed for 8–48 h.



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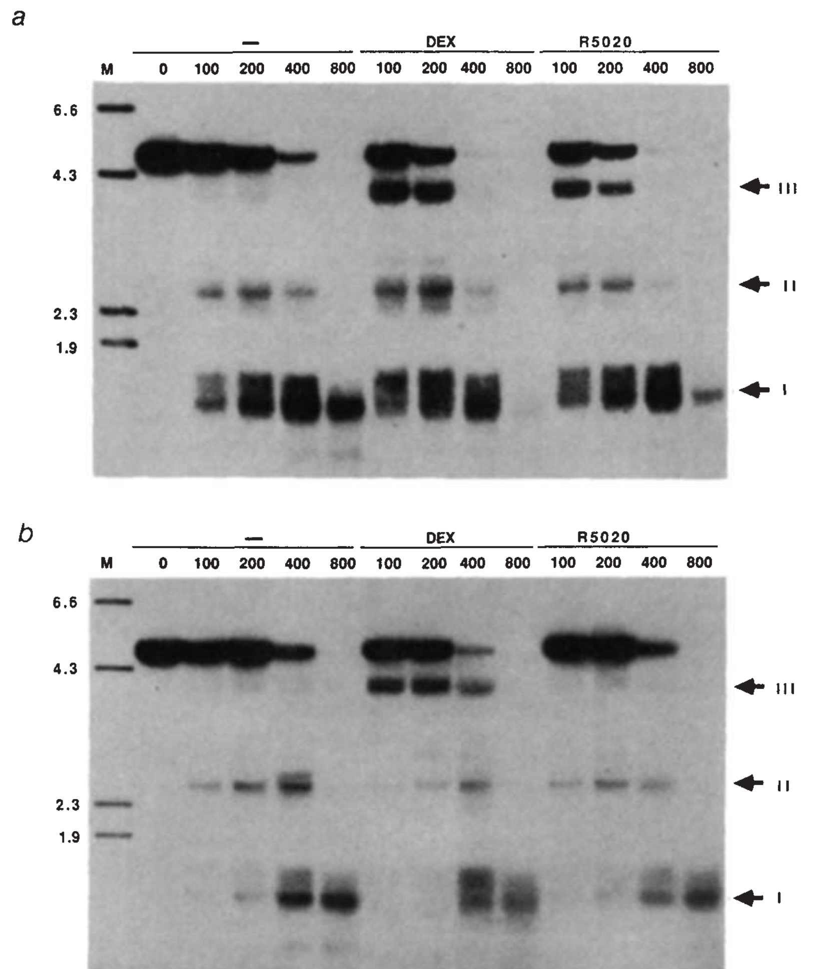


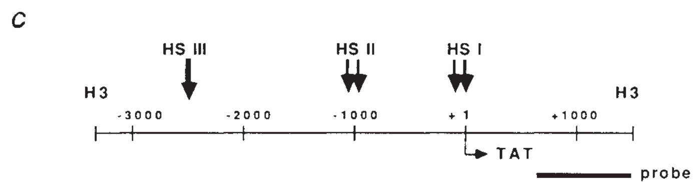


FIG. 3 R5020 induces the same changes in the chromatin structure of the TAT gene as glucocorticoids. DNase I-hypersensitive sites flanking the TAT gene were mapped in clone 1 cells (*a*) expressing the chicken progesterone receptor and in the parental Fto2B-3 cells (*b*). Clone 1 cells and Fto2B-3 cells were treated with either 10 nM R5020, 100 nM dexamethasone (DEX) or no hormone (−). Nuclei were isolated and digested with different amounts of DNase I as indicated above each lane (numbers represent units DNase I per ml). DNA was isolated and digested with *Hin*dIII (H3). Resulting fragments were analysed by Southern blotting using the probe as indicated in (*c*). Hypersensitive sites (HS) are indicated by arrows. In addition to the glucocorticoid inducible hypersensitive site (HS III) two further hypersensitive regions which serve as internal controls are visible on the autoradiographs. These correspond to the previously mapped constitutive hypersensitive sites HS I and HS II in the 5′ flanking region of the TAT gene[12,28]. (Abbreviations: DEX, dexamethasone; HS, DNase I-hypersensitive site; M, Marker, *Hin*dIII-digested and end-labelled bacteriophage λ DNA.) Mapping of the DNase I-hypersensitive site was performed as described previously[12].

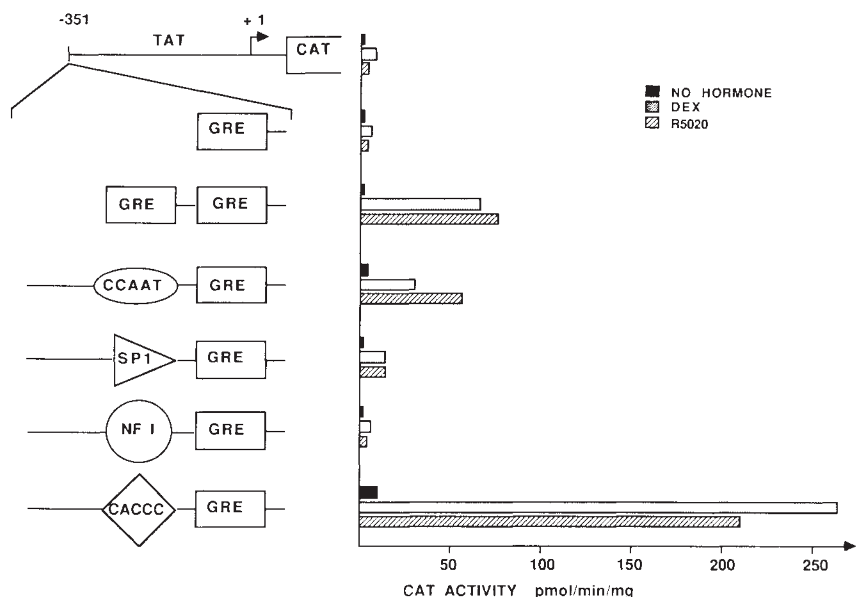


FIG. 4 Glucocorticoid receptor and progesterone receptor cooperate equally with other transcription factors. Constructs containing either one or two copies of the 15-bp GRE, or combinations of the GRE with a CCAAT-, CACCC-motif, the recognition sequence of SP1 or NFI transcription factors upstream of the TAT gene promoter driving expression of the CAT gene, were transiently transfected into clone 1 cells. After electroporation, cells were split onto plates containing either 10 nM R5020, 100 nM dexamethasone or no hormone (0.1% ethanol alone). Induction of expression was followed by determining CAT activity in the extracts. Results are presented as pmol chloramphenicol acetylated per min and per mg of extract protein. For methods see Fig. 1. The plasmid constructs have been described elsewhere[9].



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Glucocorticoids induce the formation of a DNaseI-hypersensitive site 2.5 kilobases (kb) upstream of the transcription start site in the chromatin flanking the TAT gene in Fto2B-3 cells[12]. This region binds the glucocorticoid receptor after hormone administration[13] and is responsible for glucocorticoid inducibility of the TAT gene[12]. The DNase I hypersensitive sites of the 5′-flanking region of the TAT gene were mapped in clone 1 and Fto2B-3 cells treated with dexamethasone or R5020. Both hormones induced the hypersensitive site (HS III), 2.5 kb upstream of the TAT gene, with equal effectiveness in clone 1 cells (Fig. 3*a*). Administration of R5020 to Fto2B-3 cells did not expose this hypersensitive site (Fig. 3*b*). Thus, the introduced progesterone receptor affects chromatin structure in the same way as the endogenous glucocorticoid receptor, implying that both receptors act through the same cis-regulatory elements.

Glucocorticoid and progesterone induction can be increased by cooperation of the receptors with other transcription factors[9,14,15]. Synergism between the GRE and these other cis-regulatory elements is remarkably dependent on the cell line used to analyse the recombinant constructs, indicating that the activity of the factors recognizing these elements can vary considerably between different cell lines[9]. We therefore compared the inducibility of these constructs by R5020 and dexamethasone in clone 1 cells by monitoring CAT enzymatic activity after transient transfection. Details of the constructs are described in Fig. 4 (see also ref. 9). Concordant with our previous results[9], a single 15 bp GRE did not confer inducibility on the TAT gene promoter when inserted 351 bp upstream of the transcription start site. Duplication of this GRE, or its combination with either a CACCC-, CCAAT- or SP1-sequence motif produced similar increases of CAT expression in response to either steroid hormone. No induction was detected with the construct containing a recognition sequence for nuclear factor I (NF I) combined with the GRE in this cell line. Thus, the two receptors showed a similar degree of synergism when the response elements were duplicated and both also cooperated with the same preference with other transcription factors.

In conclusion, several glucocorticoid-regulated genes in hepatoma cells respond to progestins in the same way as to glucocorticoids when the receptors for both steroid hormones are present. The progesterone receptor is expressed in only a few cell types[7]. It is not clear whether glucocorticoid receptor and progesterone receptor are both present in cells expressing genes that respond only to progesterone, such as the uteroglobin gene in rabbit uterus[11,16]. The evidence presented here strongly suggests that simply the lack of the progesterone receptor in liver prevents progestins from inducing glucocorticoid-responsive genes. Hormone-specific gene activation in specialized target cells may, however, employ further mechanisms such as selective inactivation of the hormone, as has been suggested for glucocorticoids in aldosterone target tissues[17], or differential gene activation by the A and B forms of progesterone receptor[18]. Nevertheless, our data imply that differential expression of progesterone receptor and glucocorticoid receptor is an important parameter in provoking the different physiological effects of these two classes of steroid hormones. □

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1. Evans, R. *Science* **240**, 889-895 (1988).
2. Green, S. & Chambon, P. *Trends Genet.* **4**, 309-314 (1988).
3. Beato, M. *Cell* **56**, 332-344 (1989).
4. Strähle, U. *et al. Cold Spring Harb. Symp. quant. Biol.* **53**, 835-841 (1989).
5. Strähle, U., Klock, G. & Schütz, G. *Proc. natn. Acad. Sci. U.S.A.* **84**, 7871-7875 (1987).
6. Tsai, S. *et al. Cell* **55**, 361-369 (1988).
7. Norman, A. W. & Litwack, G. in *Hormones* pp 398-643 (Academic, Orlando, 1987).
8. Gronemeyer, H. *et al. EMBO J.* **6**, 3985-3994 (1987).
9. Strähle, U., Schmid, W. & Schütz, G. *EMBO J.* **7**, 3389-3395 (1988).
10. Killary, A. M. & Fournier, R. E. K. *Cell* **38**, 523-534 (1984).
11. Ballard, P. L. *et al. Endocrinology* **94**, 998-1002 (1974).
12. Jantzen, H.-M. *et al. Cell* **49**, 29-38 (1987).
13. Becker, P. *et al. Nature* **324**, 686-688 (1986).
14. Schüle, R., Muller, M., Otsuka-Murakami, H. & Renkawitz, R. *Nature* **332**, 87-90 (1988).
15. Schüle, R., Muller, M., Kaltschmid, C. & Renkawitz, R. *Science* **242**, 1418-1420 (1988).
16. Beato, M. in *Biochemical Actions of Hormones* **14** (ed. Litwack, G.) 1-21 (Academic, Orlando, 1987).
17. Funder, J. F., Pearce, P. T., Smith, R. & Smith, I. *Science* **242**, 583-585 (1988).
18. Tora, L. *et al. Nature* **333**, 185-188 (1988).
19. Raynaud, J. P. *et al. J. Steroid Biochem.* **12**, 143-157 (1980).
20. Chen, C. & Okayama, H. *Molec. cell. Biol.* **7**, 2745-2752 (1987).
21. Miksicek, R., Borgmeyer, U. & Nowock, J. *EMBO J.* **6**, 1355-1360 (1987).
22. Chirgwin, J. M., Przybyla, A. E., MacDonald, T. J. & Rutter, W. J. *Biochemistry* **18**, 5294-5299 (1979).
23. Church, G. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1991-1995 (1984).
24. Zinn, K., DiMaio, D. & Maniatis, T. *Cell* **34**, 865-879 (1983).
25. Melton, D. *et al. Nucleic Acids Res.* **12**, 7035-7056 (1984).
26. Fort, P. *et al. Nucleic Acids Res.* **13**, 1431-1442 (1985).
27. Scherer, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7205-7208 (1982).
28. Becker, P., Renkawitz, R. & Schütz, G. *EMBO J.* **3**, 2015-2020 (1984).


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# Alteration of mouse cytochrome $P450_{coh}$ substrate specificity by mutation of a single amino-acid residue

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**As a family of structurally-related enzymes, cytochrome P450 (P450) monooxygenases exhibit paradoxical characteristics: although collectively the enzymes display a broad range of substrate specificities, individually they are characterized by a high degree of substrate and product selectivity. Mouse $P450_{15\alpha}$ and $P450_{coh}$, for example, which are expressed in female liver and male kidney cells[1,2], catalyse 15α-hydroxylation of $\Delta^4$ 3-ketone steroids, such as testosterone and 7-hydroxylation of coumarin, respectively[3–6]. In spite of their divergent catalytic activities, however, these enzymes differ by only 11 amino acids within their 494 residues[5]. To determine the structural basis of the different substrate specificities of $P450_{15\alpha}$ and $P450_{coh}$ we therefore altered each of these 11 residues by site-directed mutagenesis, expressing the mutant cytochromes in COS-1 cells. We report that the activities of both cytochromes depend critically on the identities of the amino acids at positions 117, 209 and 365 and, moreover, that a single mutation in which Phe 209 is substituted by Leu is sufficient to convert the specificity of $P450_{coh}$ from coumarin to steroid hydroxylation.**

By altering each of the 11 substituted amino acids in $P450_{coh}$ to the corresponding residue in $P450_{15\alpha}$, we obtained a series of mutant enzymes denoted C1-11 (Fig. 1). Of these, C4, C6 and C9 exhibited only 35-45% of the coumarin 7-hydroxylase activity of wild-type $P450_{coh}$ indicating that Val 117, Phe 209 and Met 365 all play important roles in the enzymatic function of the cytochrome (Table 1a). Moreover, the observation that a mutation as conservative as Val→Ala 117 profoundly affects the activity of $P450_{coh}$ implies that Val 117 is located at a particularly critical position in the active site. When all three amino acids were changed in the same mutant (C469), the coumarin 7-hydroxylase activity was completely abolished, providing further proof that these three amino acids are essential for the maximal activity of wild-type $P450_{coh}$. Western blots performed to monitor the expression of wild-type $P450_{coh}$ and its mutants, demonstrated that there was very little difference in the amounts of immunoreactive protein in the transfected cell homogenates (Fig. 2). It was concluded, therefore, that the changes in the activities of the mutants were not due to differences in the levels of expression in the transfected cells.

We also tested for increases in steroid 15α-hydroxylase activity in each mutant. As shown in Table 1*a*, only one mutant



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