

FLEEL (11) alone, FLEEL with activator ammonium sulfate, FLEEL with activator proFIX 19 (12), and FLEEL with both activators, respectively. The increases in carboxylase activity in response to different activators were consistently reproduced in separate transient-expression experiments. Microsomes isolated from permanent cell lines expressing the exogenous carboxylase showed a twofold increased carboxylase activity over the transiently expressing cells.

REFERENCES AND NOTES

1. P. A. Price, *Ann. Rev. Nutr.* **8**, 565 (1988).
2. S.-M. Wu, D. P. Morris, D. W. Stafford, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2236 (1991).
3. The bovine and human liver cDNA libraries were obtained from Stratagene.
4. The λ gt10 library was made from HEL cells which had been selected for large cells with characteristics of megakaryocytes.
5. T. Brody and J. W. Suttie, *Biochim. Biophys. Acta* **923**, 1 (1987).
6. D. M. Engelman, T. A. Steitz, A. Goldman, *Ann. Rev. Biophys. Chem.* **15**, 321 (1986).
7. S. J. Singer, *Annu. Rev. Cell Biol.* **6**, 247 (1990).
8. T. L. Carlisle and J. W. Suttie, *Biochemistry* **19**, 1161 (1980).
9. W. R. Pearson and D. J. Lipman, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2444 (1988).
10. S. Andersson, D. L. Davis, H. Dahlback, H. Jornvall, D. W. Russell, *J. Biol. Chem.* **264**, 8222 (1989).
11. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
12. proFIX 19 is an analog of the propeptide of human FIX and has the amino acid sequence AVFLD-HENANKILNRPKRY.
13. A. Hara and N. S. Radin, *Anal. Biochem.* **90**, 420 (1978).
14. J. F. Habener *et al.*, *Proc. Natl. Acad. Sci. USA* **85**, 1735 (1988).
15. T. Maniatis, E. Fritsch, J. Sambrook, *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
16. F. L. Graham and A. J. van der Eb, *Virology* **52**, 456 (1973).
17. J. M. Girardot, *J. Biol. Chem.* **257**, 15008 (1982).
18. We thank J. Ware for the human erythroleukemia cDNA library.

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Dopaminergic and Ligand-Independent Activation of Steroid Hormone Receptors

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The current view of how steroid hormone receptors affect gene transcription is that these receptors, on binding ligand, change to a state in which they can interact with chromatin and regulate transcription of target genes. Receptor activation is believed to be dependent only on this ligand-binding event. Selected steroid hormone receptors can be activated in a ligand-independent manner by a membrane receptor agonist, the neurotransmitter dopamine. In vitro, dopamine faithfully mimicked the effect of progesterone by causing a translocation of chicken progesterone receptor (cPR) from cytoplasm to nucleus. Dual activation by progesterone and dopamine was dissociable, and a serine residue in the cPR was identified that is not necessary for progesterone-dependent activation of cPR, but is essential for dopamine activation of this receptor.

STEROID HORMONE RECEPTORS ARE ligand-inducible members of a superfamily of transcription factors that also includes receptors for thyroid hormone, retinoic acid, and vitamin D₃ (1). Steroid hormone action involves entry of hormone into a cell where the hormone binds to and induces a conformational change in its cognate intracellular receptor protein (2), leading to nuclear translocation and modulation of gene expression (3). These events are believed to be dependent exclusively on hormone binding.

Steroids also modulate gene expression in neuronal tissue. Examples of steroid action

in brain include the induction of steroid aromatase by testosterone in preoptic neurons and the induction of receptors for progesterone and oxytocin by estradiol in the ventromedial hypothalamus (4). The presence of receptors for sex steroid hormones in brain raises certain biological questions. For example, what function can be ascribed to the progesterone receptors in male brain (5) or to the androgen receptors in the cerebral cortex of female brain (6)? In addition, rapid effects of steroids in neuronal tissue occur (7) that are inconsistent with the existing pathway of steroid hormone action. A derivative of adenosine 3',5'-monophosphate (cAMP), dibutyryl cAMP, can substitute for progesterone in eliciting mating behavior in female rats (8). This suggests some steroid pathways in brain may not be regulated exclusively by classical

steroid-receptor pathways but also by modified intracellular second messengers. In support of this hypothesis, 8-bromo-cAMP (8-Br-cAMP) has been demonstrated to mediate PR-dependent transcription in the absence of progesterone (9). Phosphorylation of the oncogenic derivative of the thyroid hormone receptor, erbA, is required for its function as an oncogene (10). Furthermore, the chicken ovalbumin upstream promoter (COUP) transcription factor, a member of the steroid receptor superfamily (11), can be activated by the catecholamine neurotransmitter dopamine but does not bind this compound (12). We therefore examined whether more classical members of the steroid receptor superfamily can be activated via cell membrane mechanisms such as dopaminergic or adrenergic receptor systems.

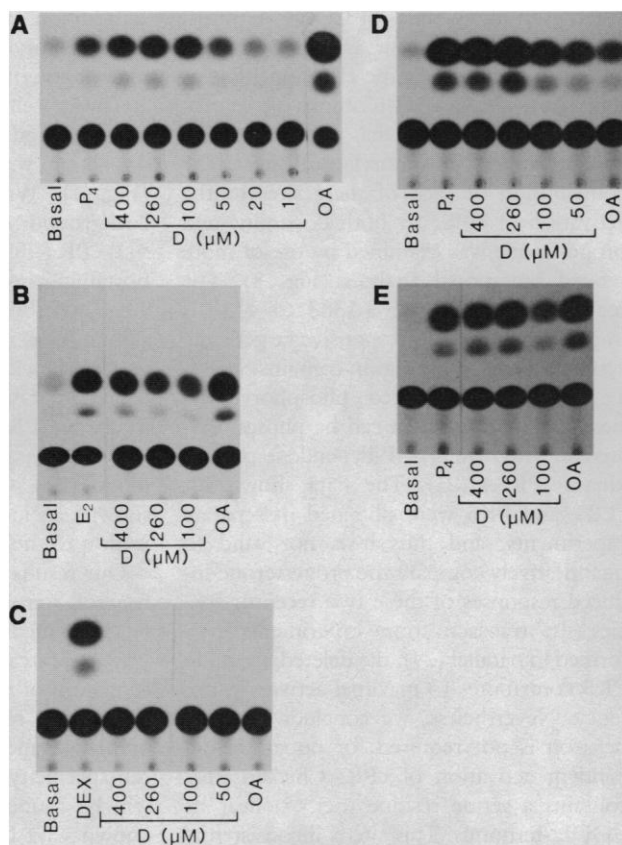
Progesterone receptor-negative monkey kidney (CV₁) cells were cotransfected with a chicken progesterone receptor A form (cPR_A) expression vector (13) and a reporter plasmid (PREtKCAT) (14) that contained a progesterone-glucocorticoid response element (PRE-GRE) upstream of the bacterial chloramphenicol acetyltransferase (CAT) gene. Treatment of these cells with progesterone or dopamine increased PR_A-mediated transcription to an equivalent degree (Fig. 1A). The response to dopamine was maximal at a concentration of 100 μ M, which is the concentration of this compound required to stimulate adenylyl cyclase activity maximally in these cells. Dopamine had no effect on CAT gene expression in cells transfected with the parent expression vector, p91023(B) and PREtKCAT (12). Dopamine does not bind to the PR (15), and there are no significant homologies between the PR sequence and published sequences for dopamine receptors. In addition, okadaic acid, an inhibitor of protein phosphatases 1 and 2A, potently stimulated PR-mediated transcription in this assay system, which implicates cellular phosphorylation pathways as a ligand-independent activation mechanism for PR (9) (Fig. 1A). The cPR_B protein (13) also activated CAT gene expression in cells treated with progesterone, dopamine, or okadaic acid (16).

The human estrogen receptor (hER) was also activated by dopamine and okadaic acid (Fig. 1B). Cells were cotransfected with an ER expression construct pSVMT-wER and an ER-responsive reporter plasmid ERE-E1bCAT (17). Dopamine induced ER-dependent transcription to a level equal to that induced by the natural ligand, estradiol. Our results with other receptors indicate that the human vitamin D receptor (hVDR) and the human thyroid hormone receptor- β (hTR β) also activate transcription from tar-

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Fig. 1. The effect of dopamine (D) on the transcriptional activity of steroid hormone receptors. Transcriptional activity was measured in monkey kidney CV₁ cells cultured in serum-free media supplemented with Nutridoma-SR (Boehringer Mannheim). Transient transfections were carried out exactly as described (9, 12). (A) The cPR_A expression vector (5 µg) (13) was cotransfected with the reporter plasmid PRE-GREtKCAT (5 µg) (14). (B) The hER expression construct (2 µg) was cotransfected with an estrogen-responsive reporter gene EREt1bCAT (5 µg) (17). (C) The hGR expression construct (5 µg) (18) was also cotransfected with PRE-GREtKCAT. (D) cPR_A was cotransfected with PRE-pOVCAT-50 (5 µg) (20) or (E) with the reporter gene pAHCAT (5 µg) (21). All transfected cells were either untreated (basal) or treated with 10⁻⁷ M of the relevant ligands for each receptor: progesterone (P₄), estradiol (E₂), or dexamethasone (DEX). Cells were treated, as described previously (12), with the indicated concentrations of dopamine and okadaic acid (OA; 5 × 10⁻⁸ M). CAT activity was determined 42 hours after transfection (35). The dopamine effect on cPR_A was observed in at least 12 separate experiments. The other results are also representative of several independent experiments (36).



get genes in response to dopamine (16). Not all steroid receptor family members were activated by dopamine. A human glucocorticoid receptor (hGR) expression vector pRShGR α (18) activated transcription from the PRE-GREtKCAT reporter gene in response to dexamethasone but was unresponsive to either dopamine or okadaic acid (Fig.

1C). Cells transfected with a human mineralocorticoid (hMR) expression construct pRShMR (19) also showed the expected increase in CAT expression in response to aldosterone or dexamethasone, but showed only a marginal response to dopamine or okadaic acid (16). Immunoblot analysis of high-salt extracts from cells transfected with

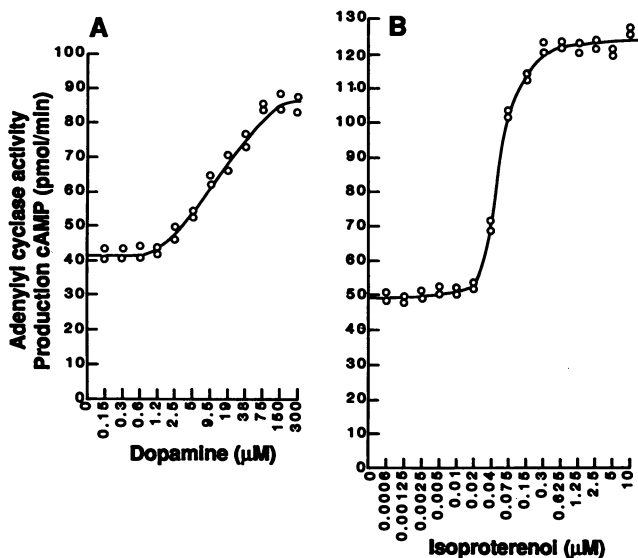
the above expression constructs did not suggest that dopamine altered intracellular receptor concentration or integrity (16).

To determine whether dopamine activation of steroid receptor-dependent target genes was promoter-specific, we tested the ability of cPR_A to induce transcription in dopamine-treated cells from reporter constructs containing either minimal or complex promoter elements. The reporter plasmid PREpOVCAT-50 (20) contains two copies of a GRE-PRE from the tyrosine amino transferase gene located 5' to the TATA box region (+43 to -50) of the chicken ovalbumin (OV) gene fused to a CAT gene. Administration of hormone or dopamine to cells cotransfected with the cPR_A expression vector and PREpOVCAT-50 resulted in 30-fold stimulation of transcription (Fig. 1D). Addition of okadaic acid also stimulated CAT gene expression, but to a lesser extent than did progesterone or dopamine. The cPR_A construct was also tested with a reporter gene (pAHCAT) (21). This construct contains the mouse mammary tumor virus long terminal repeat sequences from -1161 to +102 fused to a CAT gene. Dopamine and okadaic acid stimulated transcription of this target gene in a dose-dependent fashion to a level comparable to that induced by progesterone (Fig. 1E).

Dopamine action is mediated by its interaction with receptors coupled to guanine nucleotide-binding (G) proteins. Several dopamine receptors have been identified that belong to two main receptor subtypes, D₁ and D₂, which stimulate and inhibit adenylyl cyclase activity, respectively (22). We examined CV₁ cells for the presence of a dopamine-sensitive adenylyl cyclase (Fig. 2A). Dopamine elicited a twofold increase in cAMP in membranes prepared from these cells. These cells also exhibit a β -adrenergic receptor-linked adenylyl cyclase activity (Fig. 2B). In this case the specific β -receptor agonist isoproterenol elicited a 2.5-fold increase in cAMP.

The ability of selective D₁ or D₂ receptor agonists to mimic the dopamine activation of steroid receptor-dependent transcription was tested. Because CV₁ cells have an isoproterenol-sensitive adenylyl cyclase, the ability of α or β -adrenergic receptor agonists to induce target gene expression was also tested. Cells transfected with the cPR_A expression vector and PREtKCAT were treated with progesterone, dopamine, the selective D₁ agonist SKF38393, the selective D₂ agonist Quinpirole (LY-171555, Research Biochemicals), the β -adrenergic receptor agonist isoproterenol, and the adrenergic receptor agonists epinephrine and norepinephrine. In the absence of any ago-

Fig. 2. Dopamine-sensitive and isoproterenol-sensitive adenylyl cyclases in CV₁ cells. (A) Production of cAMP in picomoles per minute per milligram of cell protein in homogenates prepared from untransfected CV₁ cells cultured in Nutridoma-supplemented media was measured as a function of increasing dopamine concentration. Adenylyl cyclase assays were performed in duplicate for each point. (B) Production of cAMP in identical cell homogenates was measured as a function of increasing isoproterenol concentration. Duplicate assays were performed for each point. Homogenization of cells and adenylyl cyclase assays were performed as described (37).



nist, the basal CAT gene activity was a 3% conversion of substrate. The percent conversion of substrate elicited by the tested agonists were as follows: progesterone, 53%; dopamine, 55%; SKF38393, 77%; Quinpirole, 4%; isoproterenol, 3%; and epinephrine and norepinephrine, 4% (23). Thus, despite the presence of an adrenergic receptor-linked adenylyl cyclase in these cells, the activation of PR was dependent on either the addition of progesterone or stimulation of D₁ receptors.

We examined the ability of dopamine and okadaic acid to activate cPR_A mutants.

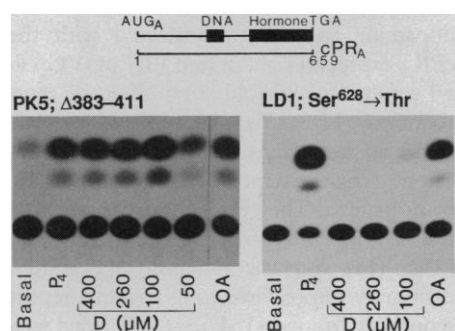


Fig. 3. Dopamine activation of cPR_A mutants. CV₁ cells were transfected as described for Fig. 1 with the reporter plasmid PRE-GREkCAT and an expression plasmid (38), containing mutated cDNAs derived from the cPR_A. The mutated expression plasmids were PK5 (24), which is a deletion mutant of a region encoding two important phosphorylation sites and LD₁ (39) in which Serine⁶²⁸ was changed to a threonine residue by oligonucleotide site-directed mutagenesis. Transfected cells were untreated (basal), or treated with progesterone dopamine (D), or okadaic acid (OA) as described for Fig. 1. CAT assays were performed 42 hours after transfection.

Analysis of gross NH₂- or COOH-terminal deletion mutants in our assay system suggested that the dopamine effect on PR was targeted to the COOH-terminus of the protein (16). This region has been shown to contain three phosphorylation sites. The contribution of each of these sites to the activation of cPR_A by both dopamine and progesterone was examined by use of more refined mutational analysis (Fig. 3). The region deleted in PK5 (Δ383 to 411) is important for steroid-responsive target gene activation (24). This region contains Ser⁴⁰², a hormonally regulated phosphorylation site, and Ser⁴⁰⁰, which can be phosphorylated in vitro by cAMP-dependent protein kinase (PKA) (25). The data shown for cPR_A and PK5 were obtained in separate experiments, and thus it is not valid to quantitatively compare the progesterone-induced responses of these two receptor species. In transactivation experiments performed in parallel (24), the deleted region in PK5 contributes to maximal activation potency. Nevertheless, we conclude that the deletion is not required for dopamine-dependent activation of cPR. Chicken PR_A contains a serine residue (Ser⁶²⁸) near its COOH-terminus. This site is inconsistently phosphorylated in vivo in oviduct slices (26). Ser⁶²⁸ was changed to a threonine by oligonucleotide site-directed mutagenesis. The resulting mutant (LD₁) was activated by progesterone and okadaic acid but was unresponsive to dopamine. A confirmatory mutation in which the serine was changed to a glycine also resulted in no activation by dopamine (16). These data point to a dual and dissociable activation mechanism for PR.

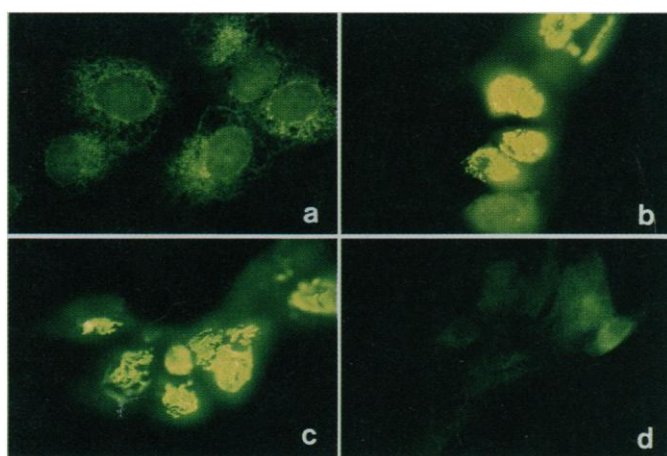
In addition, dopamine activation may result in phosphorylation or inhibition of dephosphorylation of a specific residue of PR.

The subcellular localization of PR_A in the presence and absence of progesterone or dopamine was investigated in CV₁ cells (Fig. 4). With nonimmune serum, only background staining was observed (Fig. 4D). PR_A displayed strong nuclear immunostaining in the presence of progesterone (Fig. 4B), but in the absence of hormone, staining was most evident in the cytoplasm and perinuclear region of the cytoplasm (Fig. 4A). Our results contrast with reports localizing PRs exclusively in the nucleus both in the absence and presence of hormone (27). Cells treated with dopamine showed an identical shift in receptor localization to the nucleus (Fig. 4C).

Our results extend the concept of activation of steroid receptors to a membrane receptor-mediated phosphorylation pathway or cascade. This ligand-independent activation of receptors may occur via other membrane receptors or phosphorylation pathways, and we predict that examples of cell specificity and gene-selective activation will be found in future studies. It is not known why PR is activated by dopamine, while closely related receptors are not. Perhaps dephosphorylation of GR mediates this receptor's activation (28), or GR can only be activated by its cognate ligand. Alternatively, GR may be activated via a different membrane-responsive pathway. For example, it is known that GRs are localized with high density in cell bodies and nuclei of serotonin (5-HT) and norepinephrine (NE) neurons and are involved in the regulation of the sensitivity of linked 5HT or NE receptor-coupled adenylyl cyclase systems (29).

The selectivity of the response for D₁-subtype-receptor agonists and not other membrane-associated signaling mechanisms, such as those coupled to adrenergic receptors, suggests a more complex activation mechanism than just an elevation of intracellular cAMP. This specificity could be due to a D₁ receptor-associated third messenger such as dopamine and cAMP-regulated phosphoprotein (DARPP-32). DARPP-32 is localized in D₁-containing neurons and in renal tissue (30). This protein is phosphorylated by PKA after activation of dopamine-sensitive adenylyl cyclase and, in its phosphorylated form, is a potent inhibitor of protein phosphatase 1 (31). This hypothesis would be consistent with the observation that okadaic acid activated the same steroid receptor species as did dopamine (Fig. 1). It is also possible that dopamine acts through stimulation of phospholipase-C (PL-C), as has been shown in renal cortical membranes (32). Generation of inositol-1,4,5-triphosphate and dia-

Fig. 4. Effect of dopamine on the subcellular localization of PR_A. PR-negative CV₁ cells, grown on glass cover slips, were transfected with PR_A as described in Fig. 1. Transfected cells were either untreated [(a) and (d)] or treated with 10⁻⁷ M progesterone (b) or 260 μM dopamine (c). Indirect immunofluorescence on these cells was performed as described (41) with minor modifications. Cells were fixed in 4% formaldehyde at 4°C for 10 min and then in acetone at -20°C for 10 min. Following rehydration in phosphate-buffered saline (PBS) (10 min), the cover slips were incubated in PBS containing 1% BSA and 2% normal sheep serum for 60 min at room temperature for blocking. For PR localization, cover slips were treated with a mouse monoclonal anti-PR antibody PR22 (42) [(a), (b), and (c)] or a control normal mouse IgG (d) at a 1:10 dilution for 60 min at 37°C in humidified chamber. Cover slips were then incubated in sheep antimouse IgG FITC (Amersham) at a dilution of 1:10 at 37°C for 40 min. Each incubation was followed by three washings in PBS (10 min each). Photography was performed on a Zeiss microscope (Carl Zeiss, Oberkochen, Germany) using a Kodak 160 T film with a 50X fluorar objective.



cylglycerol by such a mechanism could implicate protein kinase C as a possible transducing pathway. Or, mobilization of intracellular Ca^{2+} by inositol triphosphate could point to Ca^{2+} -calmodulin-dependent protein (CaM) kinases as the transducing factors that couple dopamine stimulation of D_1 receptors to the activation of steroid receptors. CaM kinases phosphorylate and activate the cAMP response element binding-protein (33).

Demonstration of "cross-talk" between membrane-associated receptors and intracellular steroid hormone receptors may be of biomedical significance. Dual activation of receptors might occur in situ in brain cells. It may also be possible to activate mutant forms of steroid receptors that exist in diseases (34). Finally, the finding that dopamine has direct access to the genome via this family of transcription factors may aid in understanding learning and memory processes.

REFERENCES AND NOTES

1. K. R. Yamamoto, *Annu. Rev. Genet.* **19**, 209 (1985); R. M. Evans, *Science* **240**, 889 (1988); S. Green and P. Chambon, *Trends Genet.* **4**, 309 (1988); M. Beato, *Cell* **56**, 335 (1989); B. W. O'Malley, *Mol. Endocrinol.* **4**, 363 (1990).
2. J. Gorski, D. Toft, G. Shyamala, D. Smith, A. Notides, *Recent Prog. Horm. Res.* **24**, 45 (1968); E. V. Jensen et al., *Proc. Natl. Acad. Sci. U.S.A.* **59**, 632 (1968).
3. B. W. O'Malley, W. L. McGuire, P. O. Kohler, S. Korenman, *Recent Prog. Horm. Res.* **25**, 105 (1969).
4. T. Steimer and J. B. Hutchison, *Nature* **292**, 345 (1981); B. Parsons, N. J. MacLusky, L. Krey, D. W. Pfaff, B. S. McEwen, *Endocrinology* **107**, 774 (1980); E. R. deKloet, T. A. M. Voorhuis, J. Elands, *Eur. J. Pharmacol.* **118**, 185 (1985).
5. J. D. Blaustein, H. I. Ryer, H. H. Feder, *Neuroendocrinology* **31**, 403 (1980); L. Bogic, J. L. Gerlach, B. S. McEwen, *Endocrinology* **122**, 2735 (1988).
6. S. A. Sholl and S. M. Pomerantz, *Endocrinology* **119**, 1625 (1986).
7. M. Schumacher, *Trends Neurosci.* **13**, 359 (1990).
8. C. Beyer, E. Canchola, K. Larsson, *Physiol. Behav.* **26**, 249 (1981).
9. L. A. Denner, N. L. Weigel, B. L. Maxwell, W. T. Schrader, B. W. O'Malley, *Science* **250**, 1740 (1990).
10. C. Glineur, M. Zenke, H. Beuq, J. Ghysdael, *Genes Dev.* **4**, 1663 (1990).
11. L.-H. Wang et al., *Nature* **340**, 163 (1989).
12. R. F. Power, J. P. Lydon, O. M. Conneely, B. W. O'Malley, *Science* **252**, 1546 (1991).
13. O. M. Conneely, D. M. Kettelberger, M.-J. Tsai, W. T. Schrader, B. W. O'Malley, *J. Biol. Chem.* **264**, 14062 (1989).
14. H. N. Jantzen et al., *Cell* **49**, 29 (1987).
15. N. L. Weigel and B. W. O'Malley, unpublished data.
16. R. F. Power, O. M. Conneely, B. W. O'Malley, unpublished data.
17. The human ER expression construct pSVMT-wER contains the wild-type human ER cDNA inserted downstream of a metallothionein promoter. The EREElbCAT reporter plasmid contains a fragment of the vitellogenin gene promoter (-331 to -87) upstream of an ElbTATA box linked to a CAT gene.
18. V. Giguere, S. M. Hollenberg, M. G. Rosenfeld, R. M. Evans, *Cell* **46**, 645 (1986).
19. J. L. Arriza et al., *Science* **237**, 268 (1987).
20. M. S. Bradshaw, M.-J. Tsai, B. W. O'Malley, *Mol. Endocrinol.* **2**, 1286 (1988).
21. S. K. Nordeen, B. Kuhncl, J. Lawler-Heavner, D. A. Barber, D. P. Edwards, *ibid.* **3**, 1270 (1989).
22. J. Kebabian and D. Calne, *Nature* **277**, 93 (1979); J. R. Bunzow et al., *ibid.* **336**, 783 (1988); A. Deary et al., *ibid.* **347**, 72 (1990); P. Sokoloff, B. Giros, M.-P. Martres, M.-L. Bouthenet, J.-C. Schwartz, *ibid.*, p. 146; H. H. M. Van Tol et al., *ibid.* **350**, 610 (1991); R. K. Sunahara et al., *ibid.*, p. 614.
23. R. F. Power, O. M. Conneely, B. W. O'Malley, data not shown. The results represent mean values obtained in two independent experiments.
24. A. D. W. Dobson et al., *J. Biol. Chem.* **264**, 4207 (1989).
25. L. A. Denner, W. T. Schrader, B. W. O'Malley, N. L. Weigel, *J. Biol. Chem.* **265**, 16548 (1990).
26. L. A. Denner, N. L. Weigel, B. W. O'Malley, unpublished data.
27. A. Guichon-Mantel et al., *Cell* **57**, 1147 (1989); J. A. Simental, M. Sar, M. V. Lane, F. S. French, E. M. Wilson, *J. Biol. Chem.* **266**, 510 (1991). The difference in results may reflect that, in our studies, we replaced charcoal-stripped serum with Nutridoma in the growth media 24 hours prior to transfection. This would eliminate the carryover of progestins, dopamine, and serum stimutable phosphorylation factors, among others, which may be present in trace amounts even in charcoal-stripped serum.
28. C. E. Reker, M. C. LaPointe, B. Kovacic-Milivojevic, W. J. H. Chiou, W. V. Vedeckis, *J. Steroid Biochem.* **26**, 653 (1987).
29. P. L. Mobley and F. Sulser, *Nature* **286**, 608 (1980); P. L. Mobley, D. H. Manier, F. Sulser, *J. Pharmacol. Exp. Ther.* **226**, 71 (1983); F. Sulser, *J. Clin. Psychiatr.* **10**, 13 (1986).
30. A. Aperia et al., *Am. J. Hypertens.* **3**, 11S (1990).
31. K. R. Williams, H. C. Hemmings, M. B. LoPresti, W. H. Konigsberg, P. Greengard, *J. Biol. Chem.* **261**, 1890 (1986); P. Stralfors, H. C. Hemmings, P. Greengard, *Eur. J. Biochem.* **180**, 143 (1989).
32. C. C. Felder, M. Belcher, P. A. Jose, *J. Biol. Chem.* **264**, 8739 (1989).
33. M. Sheng, M. A. Thompson, M. E. Greenberg, *Science* **252**, 1427 (1991).
34. M. R. Hughes et al., *ibid.* **242**, 1702 (1988).
35. C. M. Gorman, L. F. Moffatt, B. M. Mavard, *Mol. Cell. Biol.* **2**, 1044 (1982).
36. The experiments shown were authenticated with several batches of CV₁ cells from different sources. We observed with some batches of CV₁ cells that the dopamine response began to disappear after several successive passages for unknown reasons. In these cases, we could restore the response by changing to cells that had not been passaged as frequently.
37. M. J. Toro, E. Montaya, L. Birnbaumer, *Mol. Endocrinol.* **1**, 669 (1987).
38. G. G. Wong et al., *Science* **228**, 810 (1985).
39. The LD₁ mutant was constructed by use of oligonucleotide site-directed mutagenesis as described (13, 24). We prepared the cDNA template for mutagenesis by subcloning a 5'-Pst I-Sst I fragment of the receptor cDNA into M13 mp18. We subcloned mutants by synthesizing a second strand on the mutated M13 template with Klenow DNA polymerase, cutting with Sst I and Pst I, and ligating to an Sst I-Pst I digested plasmid (SP_A) (40) that contained the wild-type PR_A sequence. We confirmed the mutated sequence by dideoxy sequencing [F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)].
40. O. M. Conneely, B. L. Maxwell, D. O. Toft, W. T. Schrader, B. W. O'Malley, *Biochem. Biophys. Res. Commun.* **149**, 493 (1987).
41. W. C. Okulich, A. M. Savasta, L. M. Hoberg, C. Longcope, *Endocrinology* **125**, 930 (1989).
42. W. P. Sullivan, T. G. Beito, J. Proper, C. J. Krco, D. O. Toft, *ibid.* **119**, 1549 (1986).
43. We thank P. Kushner and G. L. Greene for pSVMTwER, V. Allgood and J. Cidlowski for EREElbCAT, R. M. Evans for pRShGR α , J. L. Arriza for pRShMR, and S. Nordeen for pAHCAT. We thank D. O. Toft for antibody PR22, and D. Gallup, K. Jackson, and D. Scott for technical assistance. We also thank L. Gamble and D. Scarff for help in the preparation of the manuscript and figures, respectively.

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Site-Specific Cleavage of Human Chromosome 4 Mediated by Triple-Helix Formation

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Direct physical isolation of specific DNA segments from the human genome is a necessary goal in human genetics. For testing whether triple-helix mediated enzymatic cleavage can liberate a specific segment of a human chromosome, the tip of human chromosome 4, which contains the entire candidate region for the Huntington's disease gene, was chosen as a target. A 16-base pyrimidine oligodeoxyribonucleotide was able to locate a 16-base pair purine target site within more than 10 gigabase pairs of genomic DNA and mediate the exact enzymatic cleavage at that site in more than 80 percent yield. The recognition motif is sufficiently generalizable that most cosmids should contain a sequence targetable by triple-helix formation. This method may facilitate the orchestrated dissection of human chromosomes from normal and affected individuals into megabase sized fragments and facilitate the isolation of candidate gene loci.

PYRIMIDINE OLIGODEOXYRIBONUCLEOTIDES bind in the major groove of DNA parallel to the purine Watson-

Crick strand through formation of specific Hoogsteen hydrogen bonds to the purine Watson-Crick base (1-6). Specificity is derived from thymine (T) recognition of adenine-thymine (AT) base pairs (TAT triplet); and N3-protonated cytosine (C⁺) recognition of guanine-cytosine (GC) base pairs (C + GC triplet) (1-8). The generalizability of triple-helix formation has been extended beyond purine tracts to mixed

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