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Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes

(estrogen receptor/tamoxifen/corepressor complex)

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ABSTRACT **Several lines of evidence indicate that the nuclear receptor corepressor (N-CoR) complex imposes ligand dependence on transcriptional activation by the retinoic acid receptor and mediates the inhibitory effects of estrogen receptor antagonists, such as tamoxifen, suppressing a constitutive N-terminal, Creb-binding protein/coactivator complex-dependent activation domain. Functional interactions between specific receptors and N-CoR or SMRT corepressor complexes are regulated, positively or negatively, by diverse signal transduction pathways. Decreased levels of N-CoR correlate with the acquisition of tamoxifen resistance in a mouse model system for human breast cancer. Our data suggest that N-CoR- and SMRT-containing complexes act as rate-limiting components in the actions of specific nuclear receptors, and that their actions are regulated by multiple signal transduction pathways.**

Nuclear receptors are structurally related, ligand-activated regulators of a complex array of genes involved in cell proliferation, differentiation, morphogenesis, and homeostasis (1, 2). In the absence of ligand, several nuclear receptors associate with a nuclear receptor corepressor (N-CoR) (3–6) or the related factor SMRT (silencing mediator of retinoid and thyroid receptors) (7) to mediate repression. Their regulatory function is further modulated by both physiologic and pharmacologic ligands and by the actions of various signal transduction pathways that result in ligand-independent gene activation of diverse nuclear receptor family members (8–10).

N-CoR and SMRT appear to be components of cellular complexes (4, 11, 12) containing histone deacetylases (HDACs) (13, 14) and homologs of the yeast repressor Sin3 (15, 16), which are recruited to DNA via targeting by diverse DNA-binding, site-specific transcription factors (reviewed in refs. 17 and 18). Conversely, transcriptional activation by nuclear hormone receptors requires the ligand-dependent association of a coactivator complex that includes a family of nuclear receptor coactivators (NCoAs) (19–23) and also includes the histone acetylases Creb-binding protein (CBP)/p300 (24–28) and P/CAF (29, 50).

The development of inhibitory ligands for the nuclear receptors has yielded important therapeutic treatments, among them the use of tamoxifen for endocrine therapy of breast cancer (reviewed in refs. 10 and 30). However, in certain tissues such as uterus and bone, and after long-term treatment in patients with breast cancer, tamoxifen exhibits unexplained partial agonistic activity (31). Various agents that raise intracellular cAMP levels or stimulate the ras/MAP kinase pathway can similarly cause estrogen receptor (ER) activation in the presence of tamoxifen or the absence of any activating ligand (9, 32–35). In this manuscript we show that diverse molecular strategies regulate the association of N-CoR- or SMRT-containing complexes with specific nuclear receptors, including the nature of the ligand, the levels of available N-CoR/SMRT, and the action of diverse protein kinase-dependent signaling cascades, that modulate the switch from transcriptional repression to activation.

MATERIALS AND METHODS

Protein-Interaction Assays and Cell Culture. GST interaction assays and cell extracts were performed as previously described (4) with 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) detergent instead of Nonidet P-40 for ER studies. MCF-7 cells were starved overnight in medium containing charcoal-stripped serum, treated with 10^{-6} M *trans*-hydroxytamoxifen (TOT; gift of W. Lee Kraus and Benita Katzenellenbogen), 10^{-7} M 17β-estradiol, or no ligand for 4 hr, and subsequently treated with 10 μM forskolin, 0.1 mM 8-Br-cAMP, or 50 ng/ml epidermal growth factor (EGF) for 15–30 min and lysed in CHAPS. HeLa cells were incubated in stripped medium for 6 hr before transfection with 12 μg RSV-ER or RSV-ER:S118A expression plasmid/150-mm plate. Peroxisome proliferator-activated receptor γ (PPARγ) immunoprecipitations in CV-1 cells were treated for 20 min with either EGF (50–250 ng/ml final concentration), 200 μM dopamine, or 0.1 μM phorbol 12-myristate 13-acetate, and lysed in NETN (150 mM NaCl/2 mM EDTA/20 mM Tris·HCl, pH 7.4/0.5% Nonidet P-40). Detection was performed with anti-PPARγ2 (Affinity BioReagents, Neshanic Station, NJ).

Nuclear Microinjection, Staining, and Fluorescence Microscopy. Each experiment was performed on three independent coverslips totaling approximately 1,000 cells, in triplicate. Where no experimental antibody was used, preimmune rabbit or guinea pig IgG was coinjected, allowing the unambiguous identification of injected cells in addition to serving as a preimmune control. Experimental protocol was described previously (36).

Abbreviations: ER, estrogen receptor; TOT, *trans*-hydroxytamoxifen; EGF, epidermal growth factor; RAR, retinoic acid receptor; PPARγ, peroxisome proliferator-activated receptor γ; CBP, Creb-binding protein.

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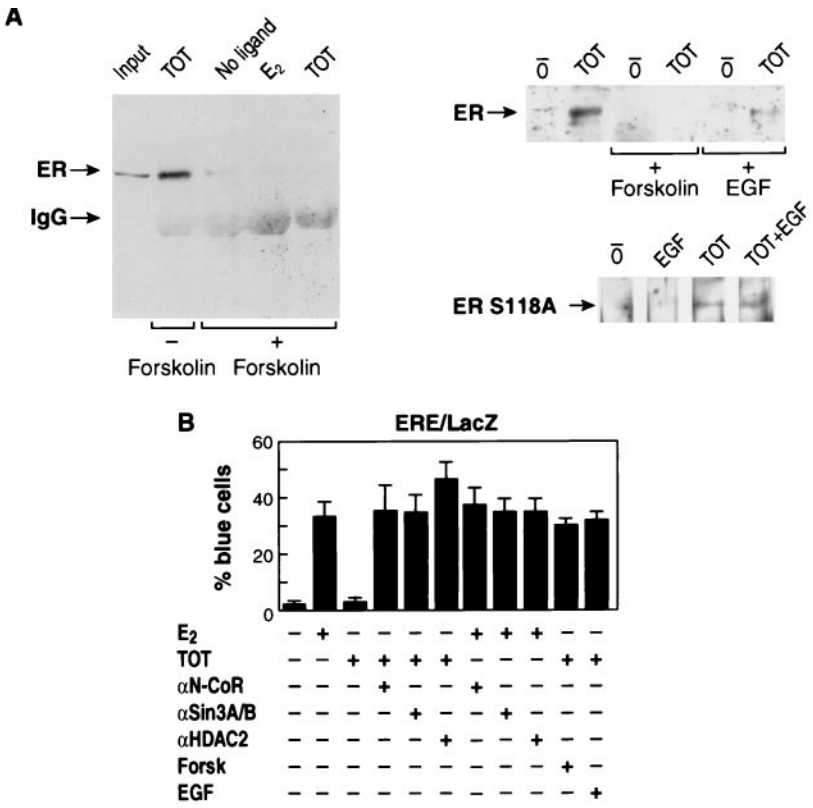

FIG. 1. (*A*) MCF-7 cells were grown in the presence or absence (Ō) of TOT and forskolin, and whole-cell extracts were immunoprecipitated with anti-N-CoR IgG and detected by using antibodies against the estrogen receptor (*Left*). ER-transfected HeLa cells were cotreated with EGF and TOT, followed by immunoprecipitation with anti-N-CoR as above. Forskolin treatment for HeLa cells is shown as a control (*Lower Right*). HeLa cells were transfected with a mutant estrogen receptor (Ser-118 → Ala), treated with the indicated combination of agents, and immunoprecipitations with N-CoR were performed as above (*Right*). (*B*) Microinjection of anti-N-CoR, anti-Sin3 (A+B), and anti-HDAC2 IgG (αN-CoR, αSin3A/B, αHDAC2) into MCF-7 cells with a LacZ reporter containing two estrogen receptor-binding elements controlling a minimal p36 promoter, in the presence or absence of 17β-estradiol or TOT. The last two columns compare these effects with those of forskolin and EGF.

Mouse Model of Tamoxifen Resistance/Tamoxifen-Stimulated Growth. MCF-7 human breast cancer cells were subcutaneously implanted into estrogen-supplemented, ovarectomized, athymic nude mice and allowed to develop as described previously (37, 38). Animal care was in accordance with institutional guidelines. Tissue was solubilized in 50 mM Tris, pH 7.8/0.2 mM EGTA/0.4 M NaCl/1 mM DTT/10% glycerol/0.1% Nonidet P-40, incubated 30 min at 4°C, and clarified by centrifugation for Western blot with anti-N-CoR (amino acids 2239–2453) using ECL (Amersham). Blots were quantitated by densitometry (Beckman DU 7 spectrophotometer) and normalized to an internal standard (MCF-7 cell extract) on each gel. Anti-actin (Chemicon) detection was performed on 1:100 dilution of extract.

RESULTS

Based on our finding that retinoic acid receptor (RAR) interacted with N-COR more strongly in the presence of antagonists (3), we tested whether ER α, which only weakly coimmunoprecipitated with N-CoR in the absence of ligand, might interact with N-CoR in the presence of an antagonist ligand. N-CoR was strongly immunoprecipitated from whole-cell extracts of MCF-7 cells (containing endogenous ER)

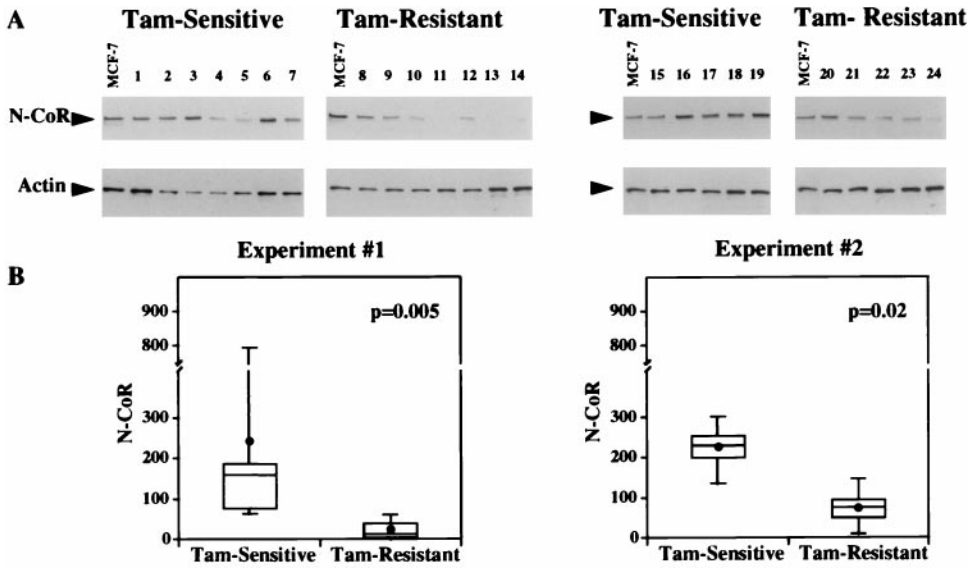

FIG. 2. A decrease in N-CoR levels correlates with the acquisition of resistance to tamoxifen by human MCF-7 breast cancer cells in a mouse model. (*A*) Western blots of whole-cell extracts from mouse tumors were normalized for total protein and probed with α-N-CoR and α-actin antibodies. Two independent sets of tumors and extracts were used. (*B*) N-CoR expression levels were quantitated by Western blot densitometry and analyzed by the Wilcoxon rank-sum test. The black circle represents the mean within each data set. The box indicates the 25th–75th percentiles; the mean (●) and median (middle line) are also shown.

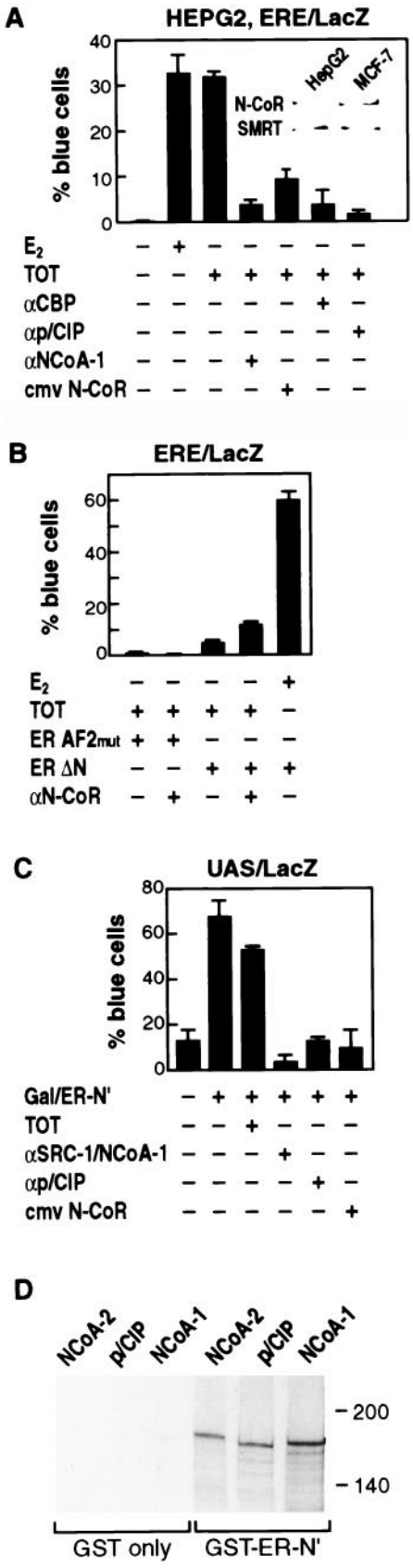

FIG. 3. TOT-bound estrogen receptor induces recruitment of the CBP coactivator complex to the N-terminal AF-1 domain. (*A*) The indicated antisera against CBP, p/CIP, or SRC-1/NCOA-1, or an expression vector for N-CoR, was microinjected as indicated into HepG2 cells with a reporter containing two estrogen response elements in front of the p36 minimal promoter. *Inset* shows Western analysis of expression levels of N-CoR and SMRT in HepG2 and MCF-7 whole-cell extract balanced for total protein loading. (*B*) Plasmids expressing mutant estrogen receptors were microinjected into Rat-1 cells with α-N-CoR IgG and a reporter containing a minimal promoter (2xEREp36LacZ). (*C*) A plasmid expressing GAL4/estrogen receptor (amino acids 1–182) was microinjected into Rat-1 cells and tested for dependence on the indicated SRC-1/NCOA-1 family members for constitutive activity. (*D*) Interaction of ^{35}S-radiolabeled proteins with GST-ER (amino acids 1–182) is shown in comparison to GST-only control lanes.

treated with the mixed anti-estrogen, TOT (Fig. 1*A*), requiring the region of N-CoR previously shown to mediate interactions with other receptors (refs. 3–7 and 39; data not shown). These ligand-specific interactions are in marked contrast to *in vitro* assays that used bacterially expressed glutathione *S*-transferase (GST) receptor and ^{35}S-labeled corepressor, in which observed ER/corepressor interactions were ligand-independent (40).

As shown in Fig. 1*A*, brief exposure of MCF7 or HeLa cells to forskolin or epidermal growth factor (EGF), agents that can switch TOT from antagonist to an agonist function (34, 41), decreased the ER/N-CoR interactions. Consistent with the observation that EGF-induced activation of the ER depends on direct phosphorylation of serine 118 (32, 35), a nonphosphorylatable mutant of ER (S118A) proved resistant to the effect of EGF on ER/N-CoR interaction (Fig. 1*A Lower Right*). Microinjection of purified IgG against N-CoR, mSin3 A/B, or HDAC2 converted TOT into an agonist in MCF-7 and Rat-1 cells (Fig. 1*B*) while exerting little effect on activity of the unliganded ER (data not shown). In the microinjection assay, treatment with forskolin or EGF also prevented the inhibitory effects of TOT.

In a mouse model system for human breast cancer that uses human MCF-7 cells growing in athymic nude mice (37, 38), prolonged treatment with tamoxifen consistently results in a transition to tamoxifen-induced tumor progression. Similarly, although tamoxifen is the most prescribed drug for the treatment of human breast cancer, all patients eventually develop drug resistance (30). Western blot densitometry analyses performed on whole-cell extracts of these tumors, normalized for total protein content and for expression levels of actin, reveal that N-CoR levels (internally normalized on each gel to identical MCF-7 control samples) declined in many of the tumors that acquired resistance to the antiproliferative effects of tamoxifen, relative to tumors retaining a response to the drug (Fig. 2*A*), whereas levels of RXR were constant between samples (data not shown). It was previously shown that the loss of tamoxifen antagonism over time is not a result of a net increase in ER levels (37). The Wilcoxon rank-sum test was used to generate a statistical summary of the results (Fig. 2*B*; $P = 0.005$ and $P = 0.02$ in two independent experiments). Because N-CoR levels were intermediate in the rapidly growing tumors from mice treated with estrogen (data not shown), the change in N-CoR protein level is not likely to be a result of cell cycle alterations accompanying the treatments.

Based on microinjection experiments using anti-CBP, p/CIP, and SRC-1/NCOA-1 IgGs, TOT-induced gene activation was found to depend on components of the coactivator complex in HepG2 cells, where TOT is a potent agonist of ER (Fig. 3*A*). Microinjection of N-CoR expression plasmid into HepG2 cells reversed TOT-induced activation, apparently overcoming an as yet unidentified mechanism that decreases binding of corepressors to TOT-bound ER.

Deletion of the N-terminal AF-1 domain of ER (ΔN75) diminished the stimulatory effect of TOT, but not 17β-estradiol, in Rat-1 cells, consistent with previously published experiments (32, 42, 43) (Fig. 3*B*), and microinjection of antibodies against N-CoR failed to activate either AF-1- or AF-2-deleted receptors (44, 45) (Fig. 3*B*). Antibodies against

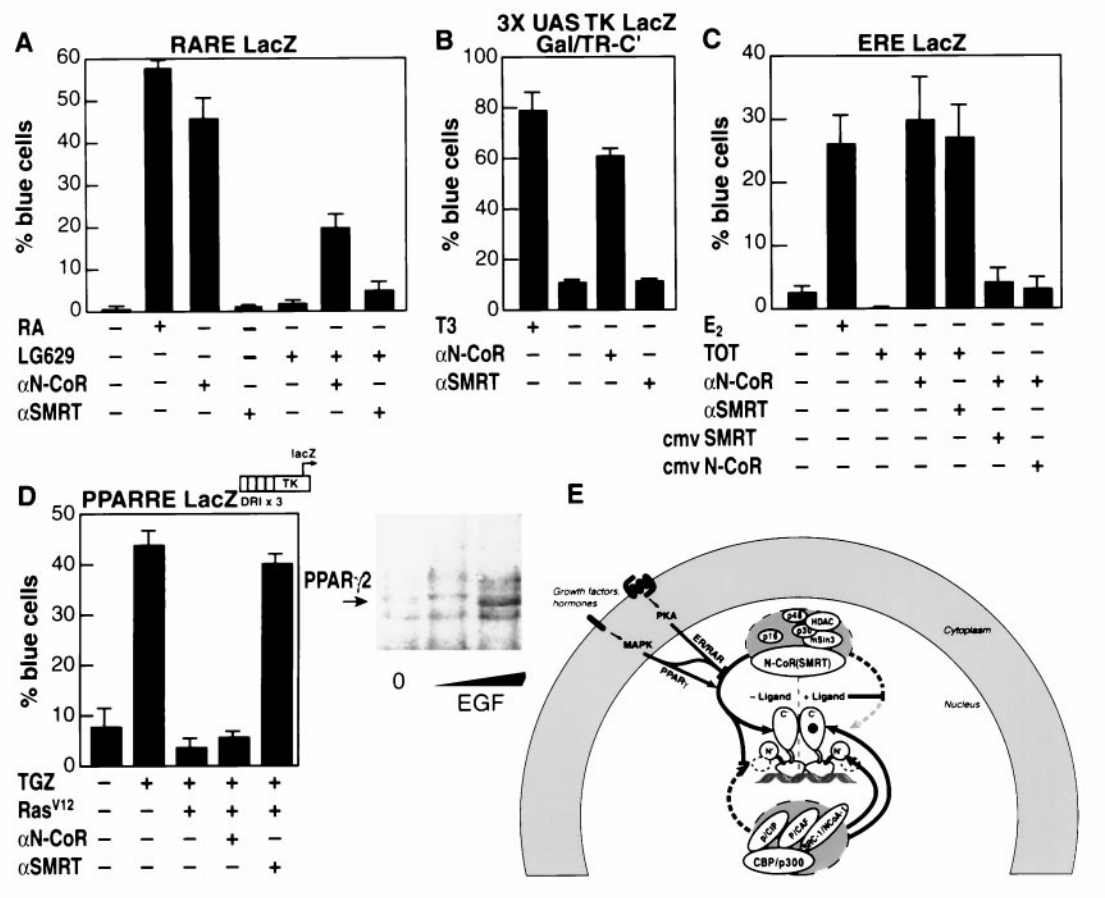

FIG. 4. N-CoR and SMRT complexes have receptor-specific roles. (*A*) A reporter under the control of retinoic acid receptor response elements was microinjected into Rat-1 cells, and effects of α-N-CoR or α-SMRT IgG were tested in the presence or absence of retinoic acid (RA) or the antagonist LG629. (*B*) A GAL fusion to the thyroid hormone receptor C terminus was microinjected, and effects of α-N-CoR or α-SMRT IgG were analyzed. (*C*) After microinjection of α-N-CoR, plasmid rescue experiments were performed as indicated. (*D*) CV-1 cells were injected with a plasmid encoding activated H-ras (val12), the indicated antisera, and a PPARγ (DR+1 site)-dependent TK promoter and treated with 1 μM troglitizone (TGZ) as indicated (*Left*). CV-1 cells were treated with increasing concentrations of EGF to stimulate MAP kinases (*Right*), and α-SMRT immunoprecipitations were performed from whole-cell extracts. Interactions were detected with antibodies against PPARγ2. (*E*) Model of regulation of nuclear receptor association with corepressor complexes. Both ligands and external signaling pathways regulate the association of specific corepressor and coactivator complexes with nuclear receptors. At least one member of a receptor homo- or heterodimer binds strongly, in the absence of ligand (or in the presence of antagonist for ER/PR), to the corepressor complex, localizing histone deacetylase activity to the promoter. This complex suppresses a constitutive N-terminal activation domain of the receptor. The corepressor complex is dismissed by agonist ligands, which allows recruitment of an acetylase-containing coactivator complex that interacts with both the receptor C-terminal AF-2 and the N-terminal AF-1 activation domains. Phosphorylation-dependent signaling pathways, initiated at the cell membrane, influence receptor activity by inhibiting the recruitment of the corepressor complex to steroid (ER/PR) and retinoid (RAR) receptors or, conversely, by stimulating its recruitment to peroxisome proliferator-activated receptor γ (PPARγ), increasing the recruitment of the coactivator complex.

the coactivators SRC-1/NCOA-1 and p/CIP abrogated the constitutive activation function of the ER N terminus, consistent with the observation that a GST/ER N-terminal fusion protein was capable of specific, but weak, interactions with SRC-1/NCOA-1, TIF2/NCOA-2, and p/CIP (Fig. 3 *C* and *D*) and a weak interaction between *in vitro* translated N-CoR and the ER N terminus.

Receptor-specific effects of N-CoR or SMRT could be demonstrated by using antibodies exhibiting no detectable cross-reactivity. Anti-N-CoR IgG, but not anti-SMRT IgG, relieved both retinoic acid and thyroid hormone receptors (Fig. 4 *A* and *B*). Additionally, anti-N-CoR IgG resulted in constitutive activation by RAR that apparently utilized the p/CIP/CBP complex required for ligand-dependent activation, as it was prevented by a p/CIP domain (amino acids 947-1084) that blocks CBP-dependent transactivation (22) (Fig. 4*A*). Anti-N-CoR was also capable of converting the RAR antagonist LG629 into a weak agonist (Fig. 4*A*). Repression by TOT-bound ER (Fig. 4*C*) and RU486-bound progesterone receptor (data not shown) was reversed by either anti-N-CoR or anti-SMRT IgG, thus converting antagonists to agonists, with either N-CoR or SMRT plasmid capable of reversing the effect of anti-N-CoR IgG (Fig. 4*C*). In contrast, PPARγ-mediated repression, stimulated by activation of the MAP kinase cascade (46), was blocked only by microinjection of antisera against SMRT (Fig. 4*D*), consistent with the observations that SMRT bound PPARγ on DNA (47) and that EGF enhanced binding of PPARγ to SMRT in whole-cell extract from EGF-treated CV-1 cells that contain endogenous PPARγ (Fig. 4*D*).

DISCUSSION

These studies reveal that the corepressor complex actually serves to impose ligand dependence on RAR, and that the anti-estrogen TOT is converted into an agonist by anti-N-CoR IgG. This suggests that either a decreasing level of N-CoR, or inhibition of corepressor binding to the receptor, might account for the ability of TOT to induce activation in specific cell types and in late-stage cancers of the breast. We have provided

evidence that both types of regulation occur *in vivo*. Activation by tamoxifen derivatives is mediated by the ER N-terminal (AF-1) domain, the function of which appears to depend on SRC-1/NCOA-1 and the p/CIP/CBP complex. In the case of ER and progesterone receptor (PR), we hypothesize that both N-CoR and SMRT complexes bind to the antagonist-bound ER C terminus and interact weakly with the constitutive ER N terminus, preventing the association of the coactivator complex. In contrast, RAR and TR appear to preferentially require N-CoR, and PPARγ selectively utilizes SMRT, perhaps reflecting preferences that are DNA- and receptor-dependent. ER and PR appear to utilize and require both corepressors in the presence of antagonists. Our observations support the model that the nature of transcriptional response to specific ligands depends on the ability of diverse signal transduction pathways to modulate the switch in nuclear receptors between a coactivator complex with histone acetylase activity and a corepressor complex with histone deacetylase activity (4, 12, 22) (Fig. 4*E*). The rate-limiting requirement of N-CoR/SMRT in estrogen receptor function suggests that there is a critical intracellular balance between the levels of N-CoR/SMRT and CBP/p300.

The data predict that a decrease in levels of N-CoR or in the affinity of the receptor for the corepressor could cause a shift in tamoxifen from antagonist to agonist, with clear implications for the use of receptor antagonists in treatment of cancers. Tamoxifen resistance (30, 31) may also be produced by decreased levels of N-CoR, overactivation of tyrosine kinase receptors or of protein kinase A (48), or by an unidentified titratable signaling pathway that regulates recruitment of N-CoR, as occurs in HepG2 cells. Because both the N-CoR corepressor complex, which plays a role in repression of transcription factors other than nuclear receptors (refs. 4, 11, and 49; R.M.L. and M.G.R., unpublished data; S. Hiebert, personal communication), and the CBP coactivator complex appear to be rate-limiting (21, 22), the regulated switch in their association that we have documented for nuclear receptors is likely to be prototypic for gene activation and repression events by many classes of transcription factors.

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1. Chambon, P. (1994) *Semin. Cell Biol.* **5,** 115–125.
2. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schultz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P. & Evans, R. M. (1995) *Cell* **83,** 835–839.
3. Hörlein, A. J., Näär, A. M., Heinzel, T., Torchia J., Gloss B., Kurokawa, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C. K. & Rosenfeld, M. G. (1995) *Nature (London)* **377,** 397–404.
4. Heinzel, T., Lavinsky, R. M., Mullen, T.-M., Soderstrom, M., Laherty, C., Torchia, J., Yang, W.-M., Brard, G., Ngo, S., Davie, J., *et al.* (1997) *Nature (London)* **387,** 43–48.
5. Zamir, I., Harding, H., Atkins, B., Horlein, A., Glass, C. K., Rosenfeld, M. G. & Lazar, M. (1996) *Mol. Cell. Biol.* **16,** 5458–5465.
6. Lee, J.-W., Choi, H.-S. Gyuris, J., Brent, R. & Moore, D. D. (1995) *Mol. Endocrinol.* **9,** 243–254.
7. Chen, J. D. & Evans, R. M. (1995) *Nature (London)* **377,** 454–457.
8. Matkovits, T. & Christakos, S. (1995) *Mol. Endocrinol.* **9,** 232–242.
9. El-Tanani, M. & Green, C. D. (1997) *Mol. Endocrinol.* **11,** 928–937.
10. Katzenellenbogen, B. S., Montano, M., Le Goff, P., Schodin, D., Kraus, W. L., Bhardwaj, B. & Fujimoto, N. (1995) *J. Steroid Biochem. Mol. Biol.* **53,** 387–393.
11. Alland, L., Muhle, R., Hou, H., Jr., Potes, J., Chin, L., Schreiber-Agus, N. & DePinho, R. A. (1997) *Nature (London)* **387,** 49–55.
12. Nagy, L., Kao, H.-Y., Chakravarti, D., Lin, R., Hassig, C., Ayer, D., Schreiber, S. & Evans, R. M. (1997) *Cell* **89,** 373–380.
13. Taunton, J., Hassig, C. & Schreiber, S. (1996) *Science* **272,** 408–411.
14. Yang, W.-M., Inouye, C., Zeng, Y., Bearss, D. & Seto, E. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 12845–12850.
15. Ayer, D. E., Lawrence, Q. A. & Eisenman, R. N. (1995) *Cell* **80,** 767–776.
16. Schreiber-Agus, N., Chin, L., Torres, R., Guida, P., Skoultchi, A. I. & DePinho, R. A. (1995) *Cell* **80,** 777–786.
17. Pazin, M. & Kadonaga, J. (1997) *Cell* **89,** 325–328.
18. Grunstein, M. (1997) *Nature (London)* **389,** 349–352.
19. Halachmi, S., Marden, E., Martin, G., MacKay, H., Abbondanza, C. & Brown, M. (1994) *Science* **264,** 1455–1458.
20. Oñate, S. A., Tsai, S., Tsai, M.-J. & O'Malley, B. W. (1995) *Science* **270,** 1354–1357.
21. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.-C., Heyman, R., Rose, D. W., Glass, C. K. & Rosenfeld, M. G. (1996) *Cell* **85,** 1–12.
22. Torchia, J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K. & Rosenfeld, M. G. (1997) *Nature (London)* **387,** 677–684.
23. Glass, C. K., Rose, D. W. & Rosenfeld, M. G. (1997) *Curr. Opin. Cell Biol.* **9,** 222–232.
24. Arias, J., Alberts, A. S., Brindle, P., Claret, F. X., Smeal, T., Karin, M., Feramisco, J. & Montminy, M. (1994) *Nature (London)* **370,** 226–229.
25. Eckner, R., Ewen, M. E., Newsome, D., Gerdes, M., DeCaprio, J. A., Lawrence, J. B. & Livingston, D. M. (1994) *Genes Dev.* **8,** 869–884.
26. Kwok, R. P., Lundblad, J. R., Chrivia, J. C., Richards, J. P., Bächinger, H. P., Brennan, R., Roberts, S. G., Green, M. R. & Goodman, R. (1994) *Nature (London)* **270,** 223–226.
27. Bannister, A. J. & Kouzarides, T. (1996) *Nature (London)* **384,** 641–643.
28. Ogryzko, V., Schlitz, R. L., Russanova, V., Howard, B. & Nakatani, Y. (1996) *Cell* **87,** 953–959.
29. Yang, X. J., Ogryzko, V., Nishikawa, J., Howard, B. & Nakatani, Y. (1997) *Nature (London)* **382,** 319–324.
30. Osborne, C. K., Elledge, R. M. & Fuqua, S. (1996) *Sci. Am.* **3,** 32–41.
31. Osborne, C. K. & Fuqua, S. A. (1994) *Breast Cancer Res. Treat.* **32,** 49–55.
32. Ali, S., Metzger, D., Bornert, J.-M. & Chambon, P. (1993) *EMBO J.* **12,** 1153–1160.
33. Ignar-Trowbridge, D., Teng, C., Ross, K., Parker, M. G., Korach, K. S. & McLachlan, J. (1993) *Mol. Endocrinol.* **7,** 992–998.
34. Smith, C., Conneely, O. & O'Malley, B. W. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6120–6124.
35. Bunone, G., Briand, P.-A., Miksicek, R. & Picard, D. (1996) *EMBO J.* **9,** 2174–2183.
36. Rose, D. W., McCabe, G., Feramisco, J. R. & Adler, M. (1992) *J. Cell. Biol.* **119,** 1405–1411.
37. Osborne, C. K., Coronado, E., Allred, D. C., Wiebe, V. & DeGregorio, M. (1991) *J. Natl. Cancer. Inst.* **83,** 1477–1482.
38. Osborne, C. K., Coronado-Heinsohn, E., Hilsenbeck, S., McCue, B., Wakeling, A., McClelland, R., Manning, D. & Nicholson, R. (1995) *J. Natl. Cancer Inst.* **87,** 46–50.
39. Seol, W., Mahon, M. J., Lee, Y. K. & Moore, D. D. (1996) *Mol. Endocrinol.* **10,** 1646–1655.
40. Smith, C., Nawaz, Z. & O'Malley, B. W. (1997) *Mol. Endocrinol.* **11,** 657–666.
41. Fujimoto, N. & Katzenellenbogen, B. S. (1994) *Mol. Endocrinol.* **8,** 296–304.
42. Metzger, D., Ali, S., Bornert, J.-M. & Chambon, P. (1995) *J. Biol. Chem.* **270,** 9535–9542.
43. McInerney, E. & Katzenellenbogen, B. S. (1996) *J. Biol. Chem.* **271,** 24172–24178.

44. McDonnell, D., Vegeto, E. & O'Malley, B. W. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10563–10567.
45. Tzukerman, M., Esty, A., Santiso-Mere, D., Danielian, P., Parker, M. G., Stein, R., Pike, J. W. & McDonnell, D. P. (1994) *Mol. Endocrinol.* **8,** 21–30.
46. Hu E., Kim, J., Sarraf, P. & Spiegelman, B. (1996) *Science* **274,** 2100–2103.
47. Zamir, I., Zhang, J. & Lazar, M. (1997) *Genes Dev.* **11,** 835–846.
48. Minton, J. P., Wisenbaugh, T. & Matthews, R. H. (1974) *J. Natl. Cancer Inst.* **53,** 283–284.
49. Dhordain, P., Albagli, O., Lin, R., Ansieau, S., Quief, S., Leutz, A., Kerckaert, J.-P., Evans, R. M. & Leprince, D. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 10762–10767.
50. Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E., Mullen, T.-M., Glass, C. K. & Rosenfeld, M. G. (1998) *Science* **279,** 703–706.