co-repressor BHC including BHC80 presumably play an important role(s) in both neuronal and non-neuronal cells. Moreover, the abundant presence of BHC80 in the nucleus of testicular germ cells (Fig. 3) implies a distinct role(s) in spermatogenesis besides neuron-specific gene repression.

An ATP-dependent chromatin-remodeling complex, NuRD, has been reported to contain DNA helicase Mi-2 that interacts directly with HDAC1 through the PHD finger domain [33,34]. The function of the PHD finger domain in 80-kDa hBHC80 (hBHC80-4) is similar to that in Mi-2; hBHC80 is capable of binding directly to each of HDAC1 and HDAC2, whereas a remarkably reduced binding to these two HDACs is found in two mutant proteins, hBHC80-M1 and hBHC80-M2, lacking the PHD finger domain in the C-terminal region (Fig. 4). Unexpectedly, 80-kDa hBHC80 also binds directly to each of three other components of BHC (Fig. 4). These results suggest that BHC80 probably serves as a scaffold protein in BHC. This probability may be supported by the fact that the RE1/NRSE-dependent transcriptional repression is abolished by overproduction of 80-kDa hBHC80 in HEK293 cells due to possible squelching of competent repressive complexes [16]. Moreover, hBHC80 exhibits the repression activity toward UAS-CMV-based reporter gene expression (Fig. 4), indicating that the recruitment of active co-repressors, including HDAC1 and HDAC2, is catalyzed by hBHC80. Thus, the C-terminal region of BHC80 containing the PHD finger domain is required for the formation of BHC mediating transcriptional repression. However, the molecular mechanism of the recruitment of other co-repressors by BHC80 still remains to be ascertained.

A major 92-kDa isoform of hBHC80 (hBHC80-6) is distinguished from another 80-kDa isoform (hBHC80-4) by reduced binding to HDAC1 and HDAC2 (Fig. 5), despite that these two hBHC80s contain the PHD finger domain (Figs. 1 and 2) and repress UAS-CMV-based gene expression (Fig. 5). Because the structural difference between the 92- and 80-kDa proteins is restricted to the replacement of the 8-residue sequence in AIF3 by the 55-residue sequence in AIF5 in the 3′-alternative region (Fig. 1), the 55-residue segment may hinder HDAC1 or HDAC2 from binding to BHC80, presumably to its PHD finger domain. It is also interesting to suppose that the hindrance is caused by an interaction of the 55-residue segment with an unknown protein(s). Both N-CoR and mSin3A have been characterized as scaffold proteins of co-repressor complexes [4,35]. However, the splicing variants of these two scaffolds have not yet been identified. Thus, this study emphasizes the possibility that alternative splicing regulates the function of scaffold protein BHC80 in BHC by altering the protein–protein interaction.

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References

[1] G.J. Narlikar, H.Y. Fan, R.E. Kingston, Cooperation between complexes that regulate chromatin structure and transcription, Cell 108 (2002) 475–487.
[2] H.M. Chan, N.B. La Thangue, p300/CBP proteins: HATs for transcriptional bridges and scaffolds, J. Cell Sci. 114 (2001) 2363–2373.
[3] R. Marmorstein, Structure of histone acetyltransferases, J. Mol. Biol. 311 (2001) 433–444.
[4] J. Ahringer, NuRD and SIN3 histone deacetylase complexes in development, Trends Genet. 16 (2000) 351–356.
[5] N. Mori, C. Schoenherr, D.J. Vandenbergh, D.J. Anderson, A common silencer element in the SCG10 and type II Na^+ channel genes binds a factor present in nonneuronal cells but not in neuronal cells, Neuron 9 (1992) 45–54.
[6] S. Schoch, G. Cibelli, G. Thiel, Neuron-specific gene expression of synapsin I. Major role of a negative regulatory mechanism, J. Biol. Chem. 271 (1996) 3317–3323.
[7] C.J. Schoenherr, A.J. Paquette, D.J. Anderson, Identification of potential target genes for the neuron-restrictive silencer factor, Proc. Natl. Acad. Sci. USA 93 (1996) 9881–9886.
[8] J.A. Chong, J. Tapia-Ramirez, S. Kim, J.J. Toledo-Aral, Y. Zheng, M.C. Boutros, Y.M. Altshuller, M.A. Frohman, S.D. Kraner, G. Mandel, REST: a mammalian silencer protein that restricts sodium channel gene expression to neurons, Cell 80 (1995) 949–957.
[9] C.J. Schoenherr, D.J. Anderson, The neuron-restrictive silencer factor (NRSF): a coordinate repressor of multiple neuron-specific genes, Science 267 (1995) 1360–1363.
[10] Z.F. Chen, A.J. Paquette, D.J. Anderson, NRSF/REST is required in vivo for repression of multiple neuronal target genes during embryogenesis, Nat. Genet. 20 (1998) 136–142.
[11] Y. Naruse, T. Aoki, T. Kojima, N. Mori, Neural restrictive silencer factor recruits mSin3 and histone deacetylase complex to repress neuron-specific target genes, Proc. Natl. Acad. Sci. USA 96 (1999) 13691–13696.
[12] J.A. Grimes, S.J. Nielsen, E. Battaglioli, E.A. Miska, J.C. Speh, D.L. Berry, F. Atouf, B.C. Holdener, G. Mandel, T. Kouzarides, The co-repressor mSin3A is a functional component of the REST-CoREST repressor complex, J. Biol. Chem. 275 (2000) 9461–9467.
[13] A. Roopra, L. Sharling, I.C. Wood, T. Briggs, U. Bachfischer, A.J. Paquette, N.J. Buckley, Transcriptional repression by neuron-restrictive silencer factor is mediated via the Sin3-histone deacetylase complex, Mol. Cell. Biol. 20 (2000) 2147–2157.
[14] M.E. Andres, C. Burger, M.J. Peral-Rubio, E. Battaglioli, M.E. Anderson, J. Grimes, J. Dallman, N. Ballas, G. Mandel, CoREST: a functional corepressor required for regulation of neural-specific gene expression, Proc. Natl. Acad. Sci. USA 96 (1999) 9873–9878.
[15] V.V. Lunyak, R. Burgess, G.G. Prefontaine, C. Nelson, S.H. Sze, J. Chenoweth, P. Schwartz, P.A. Pevzner, C. Glass, G. Mandel, M.G. Rosenfeld, Corepressor-dependent silencing of chromosomal regions encoding neuronal genes, Science 298 (2002) 1747–1752.
[16] M.A. Hakimi, D.A. Bochar, J. Chenoweth, W.S. Lane, G. Mandel, R. Shiekhattar, A core-BRAF35 complex containing

histone deacetylase mediates repression of neuronal-specific genes, Proc. Natl. Acad. Sci. USA 99 (2002) 7420–7425.
[17] K. Ohmura, N. Kohno, Y. Kobayashi, K. Yamagata, S. Sato, S. Kashiwabara, T. Baba, A homologue of pancreatic trypsin is localized in the acrosome of mammalian sperm and is released during acrosome reaction, J. Biol. Chem. 274 (1999) 29426–29432.
[18] A. Honda, K. Yamagata, S. Sugiura, K. Watanabe, T. Baba, A mouse serine protease TESP5 is selectively included into lipid rafts of sperm membrane presumably as a glycosylphosphatidylinositol-anchored protein, J. Biol. Chem. 277 (2002) 16976–16984.
[19] H. Nishimura, E. Kim, T. Fujimori, S. Kashiwabara, A. Kuroiwa, Y. Matsuda, T. Baba, The ADAM1a and ADAM1b genes, instead of the ADAM1 (fertilin α) gene, are localized on mouse chromosome 5, Gene 291 (2002) 67–76.
[20] D. Baba, S. Kashiwabara, A. Honda, K. Yamagata, Q. Wu, M. Ikawa, M. Okabe, T. Baba, Mouse sperm lacking cell surface hyaluronidase PH-20 can pass through the layer of cumulus cells and fertilize the egg, J. Biol. Chem. 277 (2002) 30310–30314.
[21] G.W. Humphrey, Y. Wang, V.R. Russanova, T. Hirai, J. Qin, Y. Nakatani, B.H. Howard, Stable histone deacetylase complexes distinguished by the presence of SANT domain proteins CoREST/kiaa0071 and Mta-L1, J. Biol. Chem. 276 (2001) 6817–6824.
[22] S. Kashiwabara, J. Noguchi, T. Zhuang, K. Ohmura, A. Honda, S. Sugiura, K. Miyamoto, S. Takahashi, K. Inoue, A. Ogura, T. Baba, Regulation of spermatogenesis by testis-specific, cytoplasmic poly(A) polymerase TPAP, Science 298 (2002) 1999–2002.
[23] J. Yanagisawa, H. Kitagawa, M. Yanagida, O. Wada, S. Ogawa, M. Nakagomi, H. Oishi, Y. Yamamoto, H. Nagasawa, S.B. McMahon, M.D. Cole, L. Tora, N. Takahashi, S. Kato, Nuclear receptor function requires a TFTC-type histone acetyl transferase complex, Mol. Cell 9 (2002) 553–562.
[24] Y. Yanagi, Y. Masuhiro, M. Mori, J. Yanagisawa, S. Kato, p300/CBP acts as a coactivator of the cone-rod homeobox transcription factor, Biochem. Biophys. Res. Commun. 269 (2000) 410–414.
[25] K. Yamagata, K. Murayama, M. Okabe, K. Toshimori, T. Nakanishi, S. Kashiwabara, T. Baba, Acrosin accelerates the dispersal of sperm acrosomal proteins during acrosome reaction, J. Biol. Chem. 273 (1998) 10470–10474.
[26] S. Kashiwabara, T. Zhuang, K. Yamagata, J. Noguchi, A. Fukamizu, T. Baba, Identification of a novel isoform of poly(A) polymerase, TPAP, specifically present in the cytoplasm of spermatogenic cells, Dev. Biol. 228 (2000) 106–115.
[27] M.B. Kumar, P. Ramadoss, R.K. Reen, J.P. Vanden Heuvel, G.H. Perdew, The Q-rich subdomain of the human Ah receptor transactivation domain is required for dioxin-mediated transcriptional activity, J. Biol. Chem. 276 (2001) 42302–42310.
[28] R. Aasland, T.J. Gibson, A.F. Stewart, The PHD finger: implications for chromatin-mediated transcriptional regulation, Trends Biochem. Sci. 20 (1995) 56–59.
[29] C. Vinson, M. Myakishev, A. Acharya, A.A. Mir, J.R. Moll, M. Bonovich, Classification of human B-ZIP proteins based on dimerization properties, Mol. Cell. Biol. 22 (2002) 6321–6335.
[30] K. Palm, N. Belluardo, M. Metsis, T. Timmusk, Neuronal expression of zinc finger transcription factor REST/NRSF/XBR gene, J. Neurosci. 18 (1998) 1280–1296.
[31] C. Koenigsberger, J.J. Chicca II, M.C. Amoureux, G.M. Edelman, F.S. Jones, Differential regulation by multiple promoters of the gene encoding the neuron-restrictive silencer factor, Proc. Natl. Acad. Sci. USA 97 (2000) 2291–2296.
[32] T. Kuwabara, J. Hsieh, K. Nakashima, K. Taira, F.H. Gage, A small modulatory dsRNA specifies the fate of adult neural stem cells, Cell 116 (2004) 779–793.
[33] J.K. Tong, C.A. Hassig, G.R. Schnitzler, R.E. Kingston, S.L. Schreiber, Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex, Nature 395 (1998) 917–921.
[34] Y. Zhang, G. LeRoy, H.P. Seelig, W.S. Lane, D. Reinberg, The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities, Cell 95 (1998) 279–289.
[35] K. Jepsen, M.G. Rosenfeld, Biological roles and mechanistic actions of co-repressor complexes, J. Cell. Sci. 115 (2002) 689–698.

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BRCA1 function mediates a TRAP/DRIP complex through direct interaction with TRAP220

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Breast cancer susceptibility gene 1 (*BRCA1*) is a tumor suppressor gene mutated in a high percentage of hereditary breast and ovarian cancers. The multifunctional BRCA1 protein acts on cell cycle control, exerting several highly specialized DNA repair processes through diverse domains. Gene regulation through its C-terminal domain (BRCT) is indispensable for BRCA1-mediated tumor suppression, suggesting the possibility that the BRCT domain interacts with co-regulator complexes. Using a biochemical approach with HeLa S3 nuclear extracts, we isolated BRCT-associated complexes and identified one of the purified components as TRAP220. We then performed interaction studies *in vivo* (co-immunoprecipitation) and *in vitro* (glutathione S-transferase pull-down assays) and showed that BRCT directly interacted with TRAP220. This *in vitro* interaction was completely abolished by BRCT point mutations typical of those found in patients with BRCA1 that lack transactivation function. BRCA1 transactivation function was dependent on TRAP220 expression level in a transient expression assay. Moreover, a cell survival assay showed that antisense TRAP220 expression to disrupt endogenous TRAP220 expression significantly reduced the survival rate potentiated by BRCA1 after DNA damage. These results suggested that a TRAP220 complex play an important role as putative co-activator complexes in BRCA1-mediated tumor suppression.
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Germ-line mutation of *BRCA1* is well known to predispose women to early onset of breast and ovarian cancer (Venkitaraman, 2002). The *BRCA1* gene encodes a relatively large protein of 1863 amino acids, and apart from an N-terminal zinc-binding RING domain and two C-terminal tandem copies of a BRCT motif, displays little similarity to other known proteins (Futreal *et al.*, 1994; Miki *et al.*, 1994). The C-terminal BRCT domain has been shown to be involved in double-stranded DNA repair and homologous recombination (Callebaut and Mornon, 1997; Moynahan *et al.*, 1999; Scully *et al.*, 1999; Zhong *et al.*, 1999). However, the major function of BRCT is thought to be as a gene regulator, mediating BRCA1 function as a tumor suppressor. This hypothesis is based on several lines of evidence, including that the autonomous transactivation function of BRCA1 was preserved in a recombinant protein consisting of the BRCT domain fused to a GAL4 DNA-binding domain (Miyake *et al.*, 2000). In addition, missense and point mutations in the BRCT domain derived from patients with inherited breast cancer result in the loss of transcriptional activity, and BRCA1 can also act as a negative regulator on some gene promoters (Chapman and Verma, 1996; Monteiro *et al.*, 1996; Zheng *et al.*, 2001; Kawai *et al.*, 2002). Reflecting the complex nature of BRCT transactivation function, this domain has already been shown to physically interact with a number of transcription factors and co-regulators (presumably in complexes), and also associate with chromatin remodeling complexes (Anderson *et al.*, 1998; Yu *et al.*, 1998; Zhang *et al.*, 1998; Yarden and Brody, 1999; Bochar *et al.*, 2000). Moreover, transcriptional squelching between BRCA1 and estrogen receptor (ER) has recently been reported (Fan *et al.*, 1999; Zheng *et al.*, 2001). As ER is a member of the nuclear receptor (NR) gene superfamily and acts as a ligand-induced transcription factor (Mangelsdorf *et al.*, 1995; Watanabe *et al.*, 2001; Yanagisawa *et al.*, 2002), limited cellular amounts of a common co-activator complex for both BRCA1 and ER could explain the transcriptional squelching phenomenon.

To better understand the BRCT transactivation function, we screened for putative transcription co-activator complexes that directly interacted with the BRCT domain using a biochemical approach. We established an affinity column whereby the BRCT domain (amino acids 1528–1863) was immobilized as a glutathione S-transferase (GST)-fusion protein. Fractions of HeLa S3 nuclear extract, presumably containing multiprotein complexes, were applied to the affinity

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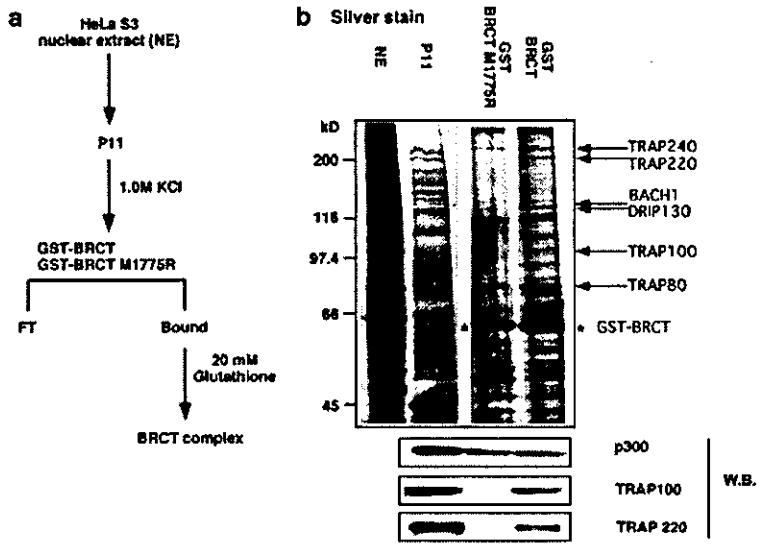

Figure 1 Affinity purification of BRCT-containing complexes (a) Purification scheme. HeLa S3 nuclear extract was fractionated using a phosphocellulose (P11) column and a GST-BRCT (amino acids 1528–1863) affinity column. The 1 M KCl P11 eluate was concentrated onto the GST-BRCT affinity column at 4°C for 10–12 h. Bound proteins were washed extensively with buffer, and subsequently eluted by buffer containing 20 mM reduced glutathione. As a negative control, purification from P11 eluates was performed using another affinity column (immobilized GST-BRCT M1775R). (b) Purified fractions were boiled, separated by electrophoresis and analysed by SDS–PAGE followed by silver staining or Western blot analysis using antibody, as shown on the right of the figure. Molecular weight standards are shown to the left of the figure. The asterisk denotes the molecular bait (GST-BRCT, GST-BRCT M1775R) after elution. Arrows show proteins identified by mass spectrometry (Yanagisawa *et al*., 2002)

column (Yanagisawa *et al*., 2002; Kitagawa *et al*., 2003), and several of the transcription co-regulators subsequently verified in the BRCT-interacting complexes by Western blotting (Figure 1b, lower panel). Western blotting of the BRCT-interacting complexes identified TRAP220 and TRAP100 (Figure 1b), which were confirmed as factors that associate with BRCT by time-of-flight mass spectrometry (TOF-MS). TRAP220 contains two LXXLL motifs, which are consensus interacting motifs and part of the core activation domain in NR ligand-binding domains (Fondell *et al*., 1999; Rachez *et al*., 1999). Thus, TRAP220 is thought to act as a major and direct interactant with ER (Yanagisawa *et al*., 2002), as well as with other NRs, as part of the common TRAP/DRIP co-activator complex (Rachez *et al*., 1999). It is therefore possible that the functional role of TRAP220 in the TRAP/DRIP complex may account for the reported transcriptional squelching between ER and BRCA1. Indeed, several other BRCT-associated proteins we identified were also TRAP/DRIP complex components, such as TRAP220, TRAP240, DRIP130, and TRAP80 (Ito *et al*., 1999). BACH1 (Cantor *et al*., 2001) and p300 (Pao *et al*., 2000) were also detected by TOF-MAS and Western blotting (Figure 1b), respectively, and have been previously shown to interact with BRCT, which confirmed the efficacy of our purification method.

To address the functional importance of the BRCT-TRAP220 interaction, a BRCT point-mutant, derived from a breast cancer patient and deficient in transcriptional activity, was used to isolate interacting complexes. As clearly shown in Figure 3b, the GST-fused BRCT point-mutant (M1775R) protein lacked the ability to retain TRAP220 on the column. This indicated that TRAP220 may function as a co-activator in the BRCA1 complex. To determine whether full-length BRCA1 protein interacted with TRAP220 in human cells, we expressed full-length BRCA1 (FLAG epitope-tagged) and/or TRAP220 (His/Myc epitope-tagged) in 293T cells. Significant expression of the tagged proteins was confirmed by Western blotting. After immunoprecipitation with anti-FLAG M2 to obtain full-length BRCA1, the immunoprecipitants were blotted with anti-Myc to identify TRAP220-containing complexes (Figure 2a). Both TRAP220 and BRCA1 were detected in cell lysate immunoprecipitates (Figure 2a), which supported the hypothesis that BRCA1 physically associates with TRAP220 in living cells. This hypothesis was further confirmed by *in vivo* association between endogenous BRCA1 and TRAP220 in MCF-7 cells expressing both proteins (Figure 2b). No such association was observed when the BRCA1 point-mutant (M1775R) was used instead of wild-type BRCA1 in the immunoprecipitation experiment (Figure 2a), as expected from the results of the column purification experiments.

To map the region of TRAP220 that interacted with BRCT, a GST pull-down assay was performed using TRAP220 deletion mutants (Figure 3a). FLAG-tagged TRAP220 fragments, abbreviated TR1, TR2, TR3, TR4 and TR5 (as described in Figure 3a), were *in vitro* translated in the presence of [^{35}S]methionine and incubated with GST-fused BRCT protein-bound resin. Only TR1 was trapped, which suggested that only the TR1 region interacted with BRCT. Interestingly, the

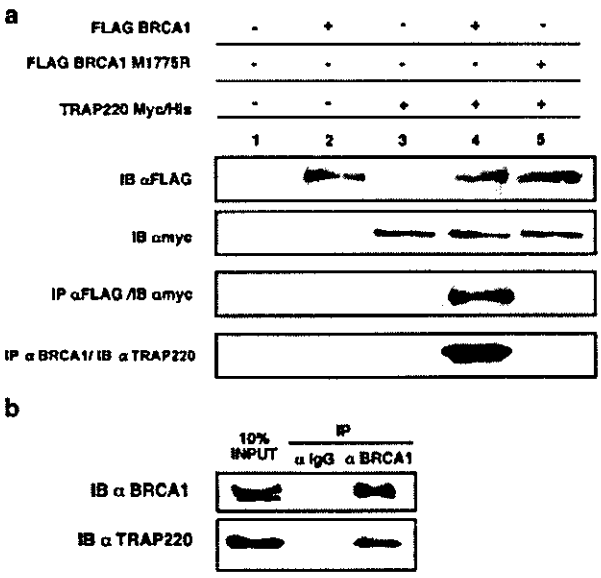

Figure 2 *In vivo* association between TRAP220 and BRCA1. (**a**) Formation of BRCA1 and TRAP220 complexes in 293T cells was analysed by co-immunoprecipitation (IP) using the anti-FLAG monoclonal M2 antibody (Sigma Aldrich) followed by immunoblotting (IB) using anti-Myc. 293T cells were transiently transfected with combinations of expression vectors as indicated. The expression of proteins in transfected cell extracts was determined by Western blot analysis using FLAG or Myc tags. (**b**) Detection of endogenous BRCA1-TRAP220 interaction by Western blotting. MCF-7 nuclear extracts were applied for immunoprecipitation with 5 μg of anti-BRCA1 (MS-BRC14-UP50, GeneTex, Inc.) and IgG, respectively. Then bound proteins in 30 μl of protein G sepharose™ 4 Fast Flow (Amersham Biosciences, NJ, USA) were detected by Western blotting. The 10% amount of the tested nuclear extracts is shown as positive control as input (Kitagawa *et al.*, 2003)

BRCT column did not retain either TR2 or TR3, which contain LXXLL motifs thought to interact with liganded NRs. Reflecting the associations between TRAP220 and wild-type or point-mutant BRCA1 as observed in our *in vivo* experiments, the point-mutations A1708E, P1749R, and M1775R that exhibit no BRCA1 transactivation function caused the loss of TRAP220 interaction *in vitro* (Figure 3b).

To examine the co-activator activity of TRAP220 toward BRCT transactivation function, a transient transfection assay was performed using a luciferase reporter plasmid driven by the adenovirus major late promoter (AdMLP) containing GAL4DBD-binding sites. A BRCT-GAL4DBD-fusion protein (GAL-BRCT) alone potently stimulated transcription (Figure 4b). TRAP220 expression in human 293T cells led to an approximately twofold increase in luciferase activity compared to GAL-BRCT alone, while such co-activation was not detected in a TRAP220 deletion mutant lacking the BRCT interacting TR1 region (amino acids 1–326, see Figure 3a) (Figure 4b). This enhancement of transactivation by TRAP220 was not observed when either GAL4 DNA-binding domain alone or GAL-BRCT mutants (A1708E, P1749R, or M1775R) were used, as expected from the *in vivo* and *in vitro* TRAP220 experiments. Consistent with these findings, antisense TRAP220 expression, that disrupted endogenous TRAP220 expression (Figure 4a), reduced the transcriptional activity of BRCT (Figure 4b). This again suggested a significant role for TRAP220 in BRCA1 transactivation function.

Finally, we then tested the significance of TRAP220 activity in the DNA damage response mediated by BRCA1. BRCA1 was transfected into HCC1937 cells (a

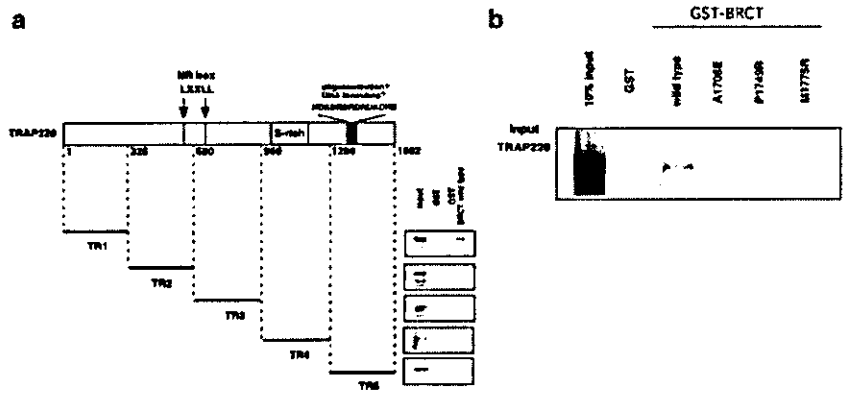

Figure 3 *In vitro* association between TRAP220 and BRCT, and mapping of the BRCT-interacting region of TRAP220. (**a**) Mapping of the BRCT-interacting region of TRAP220 using GST-BRCT and TRAP220 fragments. Bacterially expressed GST-fusion proteins immobilized on beads were used in *in vitro* pull-down assays. A schematic diagram of the structure of TRAP220 is shown. TRAP220 'TR1' (amino acids 1–326), 'TR2' (326–620), 'TR3' (620–969), 'TR4' (969–1298), and 'TR5' (1298–1582) were *in vitro* translated in the presence of [^{35}S]methionine (Amersham Pharmacia Biotech) using a TNT coupled *in vitro* translation system (Promega). Each labelled TRAP220 fragment was then incubated with either GST alone or GST-BRCT. The mixtures were washed and subjected to SDS–PAGE and analysed. Polyacrylamide gels were stained briefly with Coomassie Brilliant Blue to verify the loading of equal amounts of fusion proteins prior to drying and autoradiography (Ohtake *et al.*, 2003). (**b**) *In vitro* association of TRAP220 with BRCT or BRCT point mutants that lack transcriptional activity were performed by incubating GST, GST-BRCT, GST-BRCT A1708E, P1749R, or M1775R with *in vitro* translated TRAP220

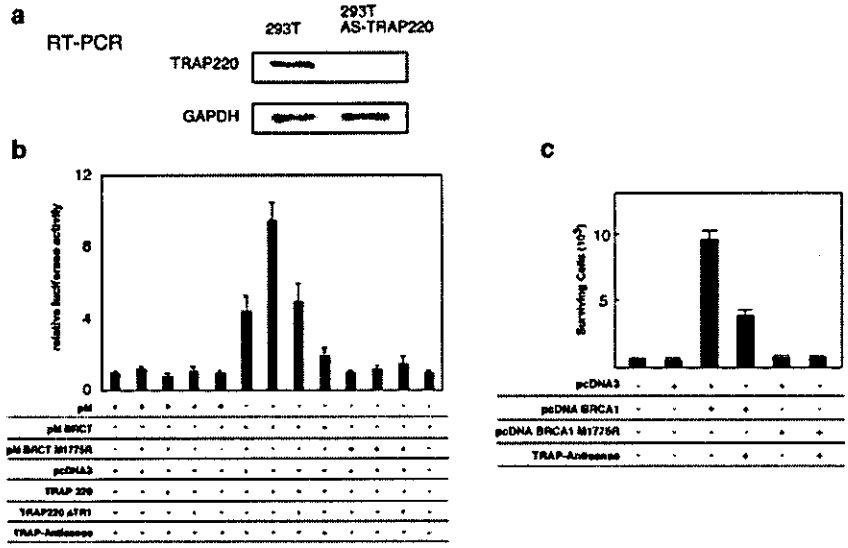

Figure 4 TRAP220 activates transcription by GAL-BRCT, while antisense TRAP220 disrupts the DNA damage response of BRCA1. (**a**) Transfection of antisense TRAP220 in 293T cells reduced TRAP220 expression as shown by RT-PCR analysis. (**b**) Transient transfection assays of GAL-BRCT and FLAG-TRAP220 using a luciferase reporter (Promega) containing the GAL4 DNA-binding site (17M8) showed specific enhancement of transcription. 293T cells were transfected with each luciferase reporter (E1b-Luc and AdMLP-Luc), pM or pM-BRCT, pRL CMV-Luc as a control of transfection efficiency, and either pcDNA3 (Invitrogen) empty expression vector, pcDNA FLAG-TRAP220, pcDNA TRAP220ΔTR1 or antisense TRAP220. Measurements of luciferase (Promega) activity were performed according to the manufacturer's instructions. Error bars indicate the standard deviation. Each experiment was repeated at least three times in triplicate (Watanabe *et al*., 2001). (**c**) HCC1937 cells were transfected with constructs based on the pcDNA3 plasmid. Cultures were treated with 0.1% MMS for 50 min, and surviving cells were counted after 8 days, as previously described by Zhong *et al*. (1999)

mutated BRCA1 cell line) that are hypersensitive to DNA damaging agents such as methylmethane sulfonate (MMS). By counting the number of surviving HCC1937 cells, we observed a protective effect of BRCA1 expression in response to DNA damage. Antisense TRAP220 expression resulted in specific deterioration in the DNA damage response potentiated by BRCA1 (Figure 4c), which verified the importance of TRAP220 in BRCA1 function.

BRCA1 is a multifunctional protein that acts as a tumor suppressor controlling gene expression, as well as a sequence-specific regulator and a co-regulator controlling DNA damage (Venkitaraman, 2002). Therefore, it can be speculated that BRCA1 acts as a platform protein that associates with a number of factors, regulators, and complexes to accomplish the diverse functions attributed to BRCA1. Indeed, discrete classes of factors and complexes involved in gene regulation and DNA repair associated with BRCA1 have been identified, and indirect associations with further related factors and complexes are supposed. Nevertheless, clear relationships between *BRCA1* gene mutation and consequent malfunctions of the identified factors and complexes remain to be established. To this end, we searched for co-activator complexes that recognized the BRCT domain, as mutations in this domain modulate BRCA1 transactivation function and are highly related with breast and ovarian cancer incidence (Humphrey *et al*., 1997; Greenman *et al*., 1998). Also, a previous report found that BRCA1 competed with ER in terms of transcriptional control via the BRCT domain, which suggested the possibility of common co-activator complexes between ER and BRCA1, presumably including the TRAP/DRIP complex already identified as an ER co-activator complex (Ito *et al*., 1999; Rachez *et al*., 1999; Yanagisawa *et al*., 2002).

In this study based on biochemical approaches (Yanagisawa *et al*., 2002; Kitagawa *et al*., 2003), we showed that a TRAP220-containing complex associated with wild-type BRCT through physical interaction with TRAP220. As TRAP220 binding of BRCA1 was abrogated *in vivo* and *in vitro* when clinically relevant BRCA1 mutants that lacked transactivation function (Chapman and Verma, 1996) were used as bait, the association between BRCT and TRAP220 complexes appears to be critical for normal BRCA1 function. This is supported by the findings that TRAP220 alone potently enhanced BRCA1 transactivation function, and that the disruption of endogenous TRAP220 by antisense TRAP220 led to a clear reduction in BRCA1-mediated DNA damage repair. This last result suggested that TRAP220-containing complexes may also play a role in the DNA damage repair function of BRCA1.

The TRAP/DRIP mediator complex was originally isolated as a co-activator complex for different classes of activators, including NRs, by several independent groups (Fondell *et al*., 1996; Rachez *et al*., 1999). Further study of the isolated complex components

revealed that the complexes formed a class of co-activator complexes that shared common major components along with limited numbers of specific factors (Gu *et al*., 1999). Combinations of these specific components generate a number of TRAP/DRIP complex subclasses (Freedman, 1999). The co-activator function of TRAP/DRIP complexes in *in vitro* transcription systems illustrates the direct link of the activator complex to the basal transcription machinery. The 220-kDa component of the complex, referred to as TRAP220/DRIP230, was identified as a subunit with unique properties in that it directly binds to NRs in a ligand-dependent manner through a region containing NR recognition motifs (LXXLL, NR box) (Ito *et al*., 1999; Rachez *et al*., 1999). Given that BRCA1 may compete with ER with respect to gene regulation, and that mutations in the BRCT region lead altered BRCA1-dependent gene regulation and enhanced tumorigenesis in estrogen-dependent cancers (presumably through modulation of ER-mediated estrogen signaling), interaction between TRAP220 and BRCA1 may account for the transcriptional squelching observed between BRCA1 and ER (Fan *et al*., 1999; Zheng *et al*., 2001).

The results of our study showed that TRAP220 bound directly to wild-type BRCA1, but not to BRCA1 mutants. Thus, breast cancer predisposition caused by genetic mutations in the BRCT domain may be due, at least in part, to insufficient interaction with TRAP complexes. In this respect, it is perhaps surprising that gene amplification or overexpression of TRAP220 is observed in some cancer cell lines (Zhu *et al*., 1999). Our transient transfection assay showed that TRAP220 enhanced BRCT-mediated transactivation function, such that the TRAP complex clearly served as a co-activator complex in the promoters of BRCA1 target genes. In conclusion, we propose that the failure of binding between BRCT and TRAP220 is a key event in cancer predisposition. Further investigations should be performed to elucidate the molecular mechanisms that underlie the formation BRCA1-TRAP complexes in normal cell growth, thereby revealing its role as a tumor suppressor in estrogen-responsive organs, especially the breast and ovary.

Abbreviations

BRCA1, breast cancer susceptibility gene 1; GST, glutathione S-transferase; CMV, cytomegalovirus; AdMLP, adenovirus major late promoter; NR, nuclear receptor.

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References

Anderson SF, Schlegel BP, Nakajima T, Wolpin ES and Parvin JD. (1998). *Nat. Genet.*, **19**, 254–256.

Bochar DA, Wang L, Beniya H, Kinev A, Xue Y, Lane WS, Wang W, Kashanchi F and Shiekhattar R. (2000). *Cell*, **102**, 257–265.

Callebaut I and Mornon JP. (1997). *FEBS Lett.*, **400**, 25–30.

Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DC, Sgroi DC, Lane WS, Haber DA and Livingston DM. (2001). *Cell*, **105**, 149–160.

Chapman MS and Verma IM. (1996). *Nature*, **382**, 678–679.

Fan S, Wang J, Yuan R, Ma Y, Meng Q, Erdos MR, Pestell RG, Yuan F, Auborn KJ, Goldberg ID and Rosen EM. (1999). *Science*, **284**, 1354–1356.

Fondell JD, Ge H and Roeder RG. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 8329–8333.

Fondell JD, Guermah M, Malik S and Roeder RG. (1999). *Proc. Natl. Acad. Sci. USA*, **96**, 1959–1964.

Freedman LP. (1999). *Cell*, **97**, 5–8.

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, Eddington K, McClure M, Frye C, Weaver-Feldhaus J, Ding W, Gholami Z, Söderkrist P, Terry L, Jhanwar S, Berchuck A, Iglehart JD, Marks J, Ballinger DG, Barrett JC, Skolnick MH, Kamb A and Wiseman R. (1994). *Science*, **266**, 120–122.

Greenman J, Mohammed S, Ellis D, Watts S, Scott G, Izatt L, Barnes D, Solomon E, Hodgson S and Mathew C. (1998). *Genes Chromosomes Cancer*, **21**, 244–249.

Gu W, Malik S, Ito M, Yuan CX, Fondell JD, Zhang X, Martinez E, Qin J and Roeder RG. (1999). *Mol. Cell.*, **3**, 97–108.

Humphrey JS, Salim A, Erdos MR, Collins FS, Brody LC and Klausner RD. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 5820–5825.

Ito M, Yuan CX, Malik S, Gu W, Fondell JD, Yamamura S, Fu ZY, Zhang X, Qin J and Roeder RG. (1999). *Mol. Cell.*, **3**, 361–370.

Kawai H, Li H, Chun P, Avraham S and Avraham HK. (2002). *Oncogene*, **21**, 7730–7739.

Kitagawa H, Fujiki R, Yoshimura K, Mezaki Y, Uematsu Y, Matsui D, Ogawa S, Unno K, Okubo M, Tokita A, Nakagawa T, Ito T, Ishimi Y, Nagasawa H, Matsumoto T, Yanagisawa J and Kato S. (2003). *Cell*, **113**, 905–917.

Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P and Evans RM. (1995). *Cell*, **83**, 835–839.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A and Skolnick MH. (1994). *Science*, **266**, 66–71.

Miyake T, Hu YF, Yu DS and Li R. (2000). *J. Biol. Chem.*, **275**, 40169–40173.

Monteiro AN, August A and Hanafusa H. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 13595–13599.

Moynahan ME, Chiu JW, Koller BH and Jasin M. (1999). *Mol. Cell.*, **4**, 511–518.

Ohtake F, Takeyama K, Matsumoto T, Kitagawa H, Yamamoto Y, Nohara K, Tohyama C, Krust A, Mimura J, Chambon P, Yanagisawa J, Fujii-Kuriyama Y and Kato S. (2003). *Nature*, **423**, 545–550.

Pao GM, Janknecht R, Ruffner H, Hunter T and Verma IM. (2000). *Proc. Natl. Acad. Sci. USA*, **97**, 1020–1025.

Rachez C, Lemon BD, Suldan Z, Bromleigh V, Gamble M, Naar AM, Erdjument-Bromage H, Tempst P and Freedman LP. (1999). *Nature*, **398**, 824–828.

Scully R, Ganesan S, Vlasakova K, Chen J, Socolovsky M and Livingston DM. (1999). *Mol. Cell.*, **4**, 1093–1099.

Venkitaraman AR. (2002). *Cell*, **108**, 171–182.

Watanabe M, Yanagisawa J, Kitagawa H, Takeyama K, Ogawa S, Arao Y, Suzawa M, Kobayashi Y, Yano T, Yoshikawa H, Masuhiro Y and Kato S. (2001). *EMBO J.*, **20**, 1341–1352.

Yanagisawa J, Kitagawa H, Yanagida M, Wada O, Ogawa S, Nakagomi M, Oishi H, Yamamoto Y, Nagasawa H, McMahon SB, Cole MD, Tora L, Takahashi N and Kato S. (2002). *Mol. Cell.*, **9**, 553–562.

Yarden RI and Brody LC. (1999). *Proc. Natl. Acad. Sci. USA*, **96**, 4983–4988.

Yu X, Wu LC, Bowcock AM, Aronheim A and Baer R. (1998). *J. Biol. Chem.*, **273**, 25388–25392.

Zhang H, Somasundaram K, Peng Y, Tian H, Bi D, Weber BL and El-Deiry WS. (1998). *Oncogene*, **16**, 1713–1721.

Zheng L, Annab LA, Afshari CA, Lee WH and Boyer TG. (2001). *Proc. Natl. Acad. Sci. USA*, **98**, 9587–9592.

Zhong Q, Chen CF, Li S, Chen Y, Wang CC, Xiao J, Chen PL, Sharp ZD and Lee WH. (1999). *Science*, **285**.

Zhu Y, Qi C, Jain S, Le Beau MM, Espinosa III R, Atkins GB, Lazar MA, Yeldandi AV, Rao MS and Reddy JK. (1999). *Proc. Natl. Acad. Sci. USA*, **96**, 10848–10853.

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TRRAP as a hepatic coactivator of LXR and FXR function

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Abstract

TBP-free TAF II-containing-type HAT complex subclasses, which contain hGCN5 HAT and TRRAP, appear to act as common coactivator complexes for nuclear receptors. However, their physiological significance with respect to each nuclear receptor remains to be established. To address this issue, we used hepatic cell lines (HepG2) with reduced endogenous TRRAP expression through antisense RNA expression or with overexpressed TRRAP or other major coactivators. The ligand-induced transactivation function of liver X receptor α (LXRα) and farnesoid X receptor/bile acid receptor reflected TRRAP expression levels, while that of PPARγ did not. A GST pull-down assay indicated that TRRAP contains two potential LXRα-interacting domains in the C-terminal and central domains. Expression of antisense TRRAP RNA in HepG2 cells abolished the ligand-induced expression of LXRα target genes. These results suggested that TRRAP plays an important role as a coactivator, presumably part of a complex, in lipid metabolism through regulation of the LXRα-mediated gene cascade in hepatic cells.

Keywords: Nuclear receptor; Lipid metabolism; TRRAP; Transcription

Lipid metabolism is controlled by a number of factors, including sequence-specific transcriptional activators. Such activators are thought to include nuclear receptors that, unlike other classes of activators, require ligand binding to evoke transactivation. Of 48 members of the human nuclear receptor family, liver X receptors (LXRα and β subtypes), FXR, and PPARs (α,β/δ and γ subtypes) appear to be physiologically important in lipid metabolism. It is thought that these nuclear receptors regulate the gene expression of lipid regulators, such as enzymes and lipid transporters, thereby controlling the cellular differentiation of fat tissue [1].

The process of ligand-induced transactivation by nuclear receptors requires distinct classes of co-regulators and associated complexes, along with chromatin remodeling complexes [2]. While the recruitment of these complexes at target gene promoters appears to be finely tuned and sequential, the timing and molecular basis of this recruitment remain to be elucidated [3]. Three classes of complexes have been documented as common coactivator complexes for nuclear receptors, two of which contain HAT, and the third being non-HAT TRAP/DRIP/SMCC complexes [4,5]. The best-characterized HAT coactivator complex contains CBP/p300 and p160 (SRC-1/TIF2/AIB1) HAT enzymes [6], along with other components such as PGC-1 [7]. The TFTC/PAF/MCC complex class HAT coactivator complex [8–12] contains hGCN5 HAT and TRRAP, which directly interacts with nuclear receptors via three LXXLL motifs.

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The significance of coactivator complexes has been verified by genetic disruption of components that directly interact with nuclear receptors. For example, gene disruption of TRAP220/DRIP205 in TRAP/DRIP complexes [13] resulted in significant defects in adipocyte differentiation and confirmed the in vitro observations of PPARγ coactivator function. In the present study, we decided to test the impact of TRRAP on nuclear receptors involved in lipid metabolism.

Materials and methods

Plasmid construction. Full-length FXR and LXRα cDNA were inserted into pcDNA3 vectors with MYC or HA epitope tags (Invitrogen). The LXRα LBD (amino acids 160–445) was cloned into pGEX4T-1 (Pharmacia Biotech). LXRE and FXRE luciferase reporters were constructed using the pGL3 basic vector containing the tk promoter (Promega). Other vectors were as previously described [12,14,15].

Cell culture and transactivation assay. HepG2 cells were maintained at 37 °C and 5% CO_2 in DMEM containing 10% fetal bovine serum (FBS). To establish stable transformants, parent HepG2 cells were transfected with pcDNA-AS-TRRAP by calcium phosphate precipitation [12] and cultured for 2 weeks in the presence of 500 μg/ml G418 to select for transformants [16]. Reduced TRRAP expression levels were confirmed by Western blotting and RT-PCR.

The following plasmids were used for transfection: 150 ng each reporter plasmid; 2 ng pRL-CMV (Promega) as a reference plasmid to allow normalization; 15 ng each receptor and coactivator expression plasmid; and pGEM carrier DNA to give a total of 200 ng DNA. Transfections were performed in 24-well plates using OPTI-MEM (Pharmacia) and Lipofectin (Invitrogen). At 6 h after transfection, 1 μM troglitazone, 10 μM CDCA, or 10 μM 22(*R*)-hydroxycholesterol was added to the medium. Cell extract preparation and dual luciferase assays were performed according to the manufacturer's protocols (Promega). Individual transfections, performed in triplicate wells, were repeated at least three times.

RNA isolation and RT-PCR. HepG2-derived cell lines were cultured in medium containing superstripped FBS for 24 h and ligand solution or DMSO (vehicle) added for an additional 24–48 h. Total RNA was then isolated using Trizol reagent (Life Technologies) according to the manufacturer's instructions. First-strand cDNA was synthesized using SuperscriptII (Promega) and amplified with specific oligonucleotide primers for ATP-binding cassette transporter A1 and G1 (ABCA1, ABCG1), stearoyl-CoA desaturase (SCD), and high density lipoprotein binding protein (HBP), TRRAP, and GAPDH as previously described [12,17]. Up to 30 cycles of amplification were performed, with each cycle consisting of 96 °C for 30 s, 60 °C for 45 s, and 72 °C for 90 s.

GST pull-down assay. GST-fused proteins were expressed in *Escherichia coli* and purified on glutathione–Sepharose beads as previously described [15]. In vitro translated TRRAP deletion mutant proteins labeled with [^{35}S]methionine were incubated with the beads in NET-N buffer (0.5% Nonidet P-40, 20 mM Tris–HCl (pH 7.5), 200 mM NaCl, and 1 mM EDTA containing 1 mM phenyl methylsulfonyl fluoride). Bound proteins were eluted and analyzed by 10% SDS–PAGE.

Results and discussion

To test the coactivator function of TRRAP on nuclear receptors in hepatocytes, HepG2 cells were stably transfected with an antisense expression vector that consisted of TRRAP cDNA cloned into the CMV expression vector in the antisense orientation (AS-TRRAP) [12]. AS-TRRAP-transfected HepG2 cells looked normal and retained the same proliferative ability as parental HepG2 cells (data not shown). Using TRRAP-HepG2 transformants, we examined the ligand-dependent transactivation function of the nuclear receptors LXRα, FXR, and PPARγ, as these receptors are known to regulate the gene expression of enzymes that control lipid metabolism in hepatocytes. A significant reduction in the ligand-dependent transactivation function of LXRα and FXR, but not PPARγ was observed (Fig. 1A). To further explore this receptor selectivity of TRRAP coactivator function, TRRAP and other coactivators (PGC-1, SRC-1, and TRAP220) were overexpressed and nuclear receptor coactivation was examined. As shown in Fig. 1B, PGC-1, SRC-1, and TRAP220 overexpression weakly enhanced the ligand-induced transactivation function of LXRα in HepG2 cells. Interestingly, TRRAP overexpression strongly potentiated the ligand-induced transactivation function of both FXR and LXRα (Fig. 1B, lanes 3, 4). In contrast, ligand-induced PPARγ function in HepG2 cells was enhanced mainly by PGC-1, SRC-1, and TRAP220. Thus, TRRAP appeared to act as the major coactivator of LXRα and FXR in hepatocytes, such that TRRAP exhibited stronger coactivator activity on these nuclear receptors than the other coactivators.

Ligand-dependent coactivators, such as the p160 family and TRRAP, are known to bind to nuclear receptors via the amino acid motif LXXLL [18]. TRRAP contains 10 LXXLL motifs, with the three central LXXLL motifs mapped as the direct interface for estrogen-induced association with human ERα [12]. To map the TRRAP interacting region for LXRα, we examined the direct and ligand-dependent interaction between TRRAP and LXRα using a GST pull-down assay with TRRAP deletion mutants (amino acids m1, 1–580; m2, 581–900; m3, 901–1394; m4, 1395–1890; m5, 1891–2637; m6, 2638–3261; and m7, 3262–3831) expressed in *E. coli* as GST-fusion proteins (Fig. 2A). As shown in Fig. 2B, the ligand binding domain of LXRα exhibited ligand-dependent interaction with TRRAP via amino acid regions 581–900 and 901–1394, which contain five LXXLL motifs. This result indicated that the TRRAP interaction domain for LXRα was different to that for ERα, which is located at amino acids 984–1214 and contains three LXXLL motifs [12]. Moreover, the C-terminal domain of TRRAP, similar to an ATM domain, also physically associated with LXRα in a ligand-independent manner. This suggested that the mode of TRRAP binding to LXRα may also be different to that of ERα.

LXRα regulates cholesterol metabolism and/or efflux by directly controlling target gene expression. Therefore, we examined the expression levels of several LXRα tar-

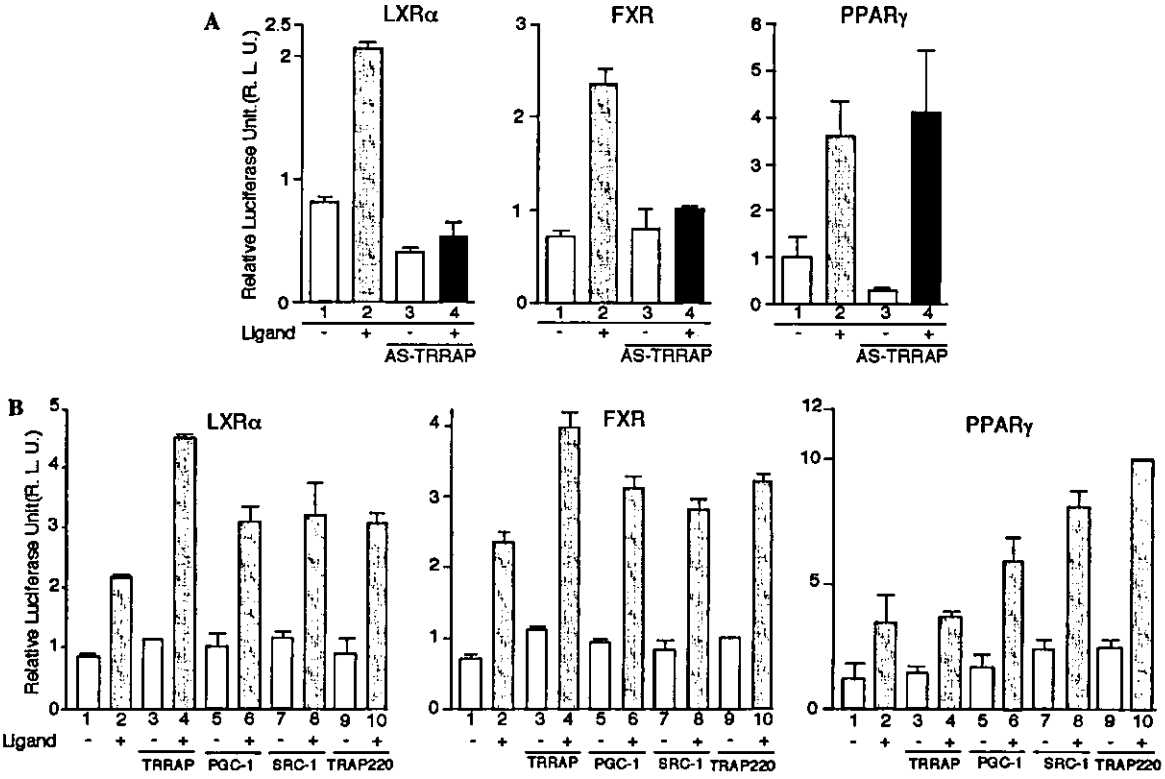

Fig. 1. TRRAP selectively activates nuclear receptor function in HepG2 cells. (A) Parental and AS-TRRAP HepG2 cells were transfected with nuclear receptor expression vectors and luciferase reporter plasmids containing the appropriate response elements. Following transfection, cells were treated for 36 h with each ligand (100 μM CDCA for FXR, 10 μM 22(*R*)-hydroxycholesterol for LXR, and 1 μM troglitazone for PPARγ). Intact and empty vector-transfected HepG2 cells did not differ in the pattern of transactivation (data not shown). Data represent means ± SD of three individual transfections. (B) Potentiation of ligand-dependent LXRα and PPARγ function by coactivators. HepG2 cells were co-transfected with reporter constructs LXRE-tk-luc, FXRE-tk-luc, or PPRE-tk-luc and with LXRα, FXR, or PPARγ expression vectors, respectively, and coactivator expression vectors as indicated.

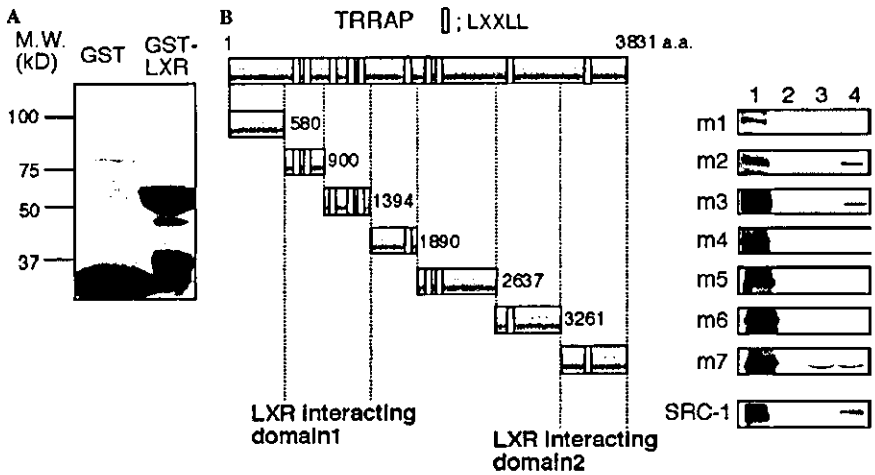

Fig. 2. The C-terminal and central domains of TRRAP interact with LXRα. (A) GST and GST-LXRα proteins expressed in *E. coli*. (B) GST pull-down assays were performed with ^{35}S-labeled TRRAP deletion mutants in the presence of GST or GST-LXRα as indicated. 22(*R*)-hydroxycholesterol was added to the binding reaction to a final concentration of 10 μM. SRC-1 was used as a positive control. 1, 10% input; 2, incubated with GST; 3, incubated with GST-LXRα without 22(*R*)-hydroxycholesterol; and 4, incubated with both GST-LXRα and 22(*R*)-hydroxycholesterol.

get genes, ATP-binding cassette transporter A1 and G1 (ABCA1 and ABCG1), stearoyl-CoA desaturase (SCD), and high density lipoprotein binding protein (HBP), in wild-type and AS-TRRAP-HepG2 cells treated with the LXRα ligand 22(*R*)-hydroxycholesterol, known to induce these genes [17]. After 24 h treatment with 100 μM 22(*R*)-hydroxycholesterol, gene mRNA levels were estimated in wild-type and AS-TRRAP-HepG2 cells by RT-PCR using the TRRAP and GAPDH genes as controls. As shown in Fig. 3, basal ABCA1, ABCG1, SCD, and HBP mRNA levels were reduced in AS-TRRAP-HepG2 cells (lane 3), but this decrease was abrogated after treatment with 22(*R*)-hydroxycholesterol (lane 4). Interestingly, TRRAP gene expression was also induced by 22(*R*)-hydroxycholesterol, although it was unclear whether this induction was due to direct or indirect ligand effects.

Nuclear receptors require a number of nuclear complexes, including chromatin remodeling complexes and co-regulators, for transcriptional regulation (see [19,20] for review). Recent observations from ChIP analyses revealed that complexes are recruited to nuclear receptors bound to promoters in different modes, and that the timing of recruitment is distinct according to the complex [3]. These findings indicate that gene regulation by nuclear receptors is a finely organized process of association between nuclear factors and complexes. Moreover, given the tissue-specific and promoter-dependent functions of nuclear receptors, as yet unidentified factors and complexes presumably support the ligand-dependent transcriptional control exerted by nuclear receptors. It is likely that the preference for functional association between particular nuclear receptors and such associating factors enables receptor-specific gene regulation within a single cell [2]. In the present study, we characterized TRRAP function in HepG2 stable transformants that expressed TRRAP antisense RNA

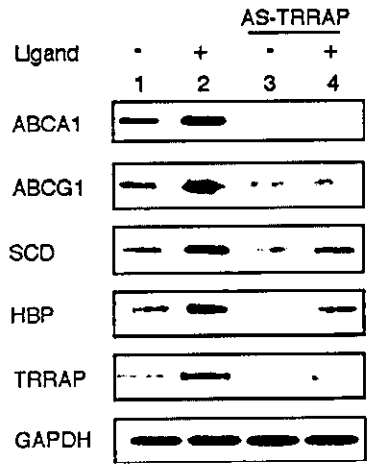

Fig. 3. TRRAP regulates LXRα-induced gene expression in HepG2 cells. Cells were treated as described, and then total RNA was isolated and analyzed by RT-PCR using the indicated primers [12,17].

by examining nuclear receptors that control endocrine and lipid metabolism. We found that of the coactivators tested, TRRAP most potently enhanced the ligand-induced transcription function of LXRα and FXR, but not PPARγ. Mapping the TRRAP interacting domain for LXRα indicated that the mode of ligand-induced TRRAP/LXRα association was different compared to the TRRAP/ERα association, even though in both cases TRRAP-containing complexes appear to serve as a significant coactivator. As TRRAP-containing complexes make up several subclasses with different combinations of specific components, it is unclear at present whether the TRRAP complex for LXRα/FXR is the same as the one for ERα. Biochemical identification of TRRAP complex components is clearly required to better understand LXRα/FXR function in hepatic cells.

As a number of known coactivators appear to potentiate the functions of many nuclear receptors, it is unclear which coactivator is physiologically significant in supporting the expression of LXRα target genes involved in cholesterol metabolism and/or efflux (including ABCA1, ABCG1, SCD, and HBP) in the liver. Given recent reports that some nuclear receptor coactivators serve as tissue-specific co-regulators [13], the identification of liver-specific coactivators for LXRα/FXR is an important step in understanding the molecular mechanisms of cholesterol and bile acid homeostasis regulation.

In HepG2 cells, SRC-1 was less effective in activating the ligand-induced transactivation function of LXRα than of PPARγ. In human embryonic kidney 293T cells, the SRC-1/p300 HAT complex enhances the ligand-dependent transactivation function of LXRα [21]. However, SRC-1 and TIF2 knockout mice do not exhibit impaired hepatic function, despite the finding of altered adipogenesis in these mice [22]. Thus, it is unlikely that SRC-1 and TIF2 activate the ligand-induced transcription function of LXRα/FXR in the liver. TRAP220 also showed reduced enhancement of LXRα/FXR ligand-dependent transcription function compared to PPARγ. Moreover, while TRAP220 knockout mice showed reduced PPARγ function and adipogenesis, no liver defects were observed [13,23]. This also suggests that TRAP220 complexes do not act as coactivators for LXRα/FXR. Although PGC-1 can potentiate LXRα transcription function, the relationship between PGC-1 and cholesterol efflux is still uncertain. PGC-1 is a coactivator that regulates energy homeostasis and gluconeogenesis in the liver through nuclear receptors and other transcriptional factors [24,25]. PGC-1 appears to bind to p160 family members through its N-terminal activation domain and physically associates with nuclear receptors through an LXXLL motif in its N-terminal domain. Interaction between PGC-1 and the DRIP/TRAP complex has also been reported [26,27], which suggests that PGC-1 may act as a tether protein, associating with both DRIP/TRAP non-HAT and p160/p300 HAT

complexes. However, as these complexes are dispensable for LXRα/FXR function in the liver, it is unlikely that PGC-1 plays a central role in regulating LXRα/FXR function in the liver.

Our results suggested that TRRAP regulates cholesterol metabolism and/or efflux and bile acid metabolism in hepatic cells through LXRα/FXR function, and fatty acid oxidation and glucose homeostasis through PPARγ function. Since TRRAP knockout mice are embryonic lethal [28], liver-specific gene disruption of TRRAP in adult mice will be necessary to verify the apparent function of TRRAP in cholesterol metabolism. Most importantly, the biochemical identification of TRRAP complex components remains to be investigated.

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References

[1] M.L. Fitzgerald, K.J. Moore, M.W. Freeman, Nuclear hormone receptors and cholesterol trafficking: the orphans find a new home, J. Mol. Med. 80 (2002) 271–281.

[2] H. Kitagawa, R. Fujiki, K. Yoshimura, Y. Mezaki, Y. Uematsu, D. Matsui, S. Ogawa, K. Unno, M. Okubo, A. Tokita, T. Nakagawa, T. Ito, Y. Ishimi, H. Nagasawa, T. Matsumoto, J. Yanagisawa, S. Kato, The chromatin-remodeling complex WINAC targets a nuclear receptor to promoters and is impaired in Williams syndrome, Cell 113 (2003) 905–917.

[3] R. Metivier, G. Penot, M.R. Hubner, G. Reid, H. Brand, M. Kos, F. Gannon, Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter, Cell 115 (2003) 751–763.

[4] J.D. Fondell, H. Ge, R.G. Roeder, Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex, Proc. Natl. Acad. Sci. USA 93 (1996) 8329–8333.

[5] C. Rachez, B.D. Lemon, Z. Suldan, V. Bromleigh, M. Gamble, A.M. Naar, H. Erdjument-Bromage, P. Tempst, L.P. Freedman, Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex, Nature 398 (1999) 824–828.

[6] S.A. Onate, S.Y. Tsai, M.J. Tsai, B.W. O'Malley, Sequence and characterization of a coactivator for the steroid hormone receptor superfamily, Science 270 (1995) 1354–1357.

[7] P. Puigserver, Z. Wu, C.W. Park, R. Graves, M. Wright, B.M. Spiegelman, A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis, Cell 92 (1998) 829–839.

[8] E. Martinez, T.K. Kundu, J. Fu, R.G. Roeder, A human SPT3-TAFII31-GCN5-L acetylase complex distinct from transcription factor IID, J. Biol. Chem. 273 (1998) 23781–23785.

[9] V.V. Ogryzko, T. Kotani, X. Zhang, R.L. Schlitz, T. Howard, X.J. Yang, B.H. Howard, J. Qin, Y. Nakatani, Histone-like TAFs within the PCAF histone acetylase complex, Cell 94 (1998) 35–44.

[10] E. Wieczorek, M. Brand, X. Jacq, L. Tora, Function of TAF(II)-containing complex without TBP in transcription by RNA polymerase II, Nature 393 (1998) 187–191.

[11] M. Brand, K. Yamamoto, A. Staub, L. Tora, Identification of TATA-binding protein-free TAFII-containing complex subunits suggests a role in nucleosome acetylation and signal transduction, J. Biol. Chem. 274 (1999) 18285–18289.

[12] J. Yanagisawa, H. Kitagawa, M. Yanagida, O. Wada, S. Ogawa, M. Nakagomi, H. Oishi, Y. Yamamoto, H. Nagasawa, S.B. McMahon, M.D. Cole, L. Tora, N. Takahashi, S. Kato, Nuclear receptor function requires a TFTC-type histone acetyl transferase complex, Mol. Cell 9 (2002) 553–562.

[13] K. Ge, M. Guermah, C.X. Yuan, M. Ito, A.E. Wallberg, B.M. Spiegelman, R.G. Roeder, Transcription coactivator TRAP220 is required for PPAR gamma 2-stimulated adipogenesis, Nature 417 (2002) 563–567.

[14] Y. Kodera, K. Takeyama, A. Murayama, M. Suzawa, Y. Masuhiro, S. Kato, Ligand type-specific interactions of peroxisome proliferator-activated receptor gamma with transcriptional coactivators, J. Biol. Chem. 275 (2000) 33201–33204.

[15] M. Suzawa, I. Takada, J. Yanagisawa, F. Ohtake, S. Ogawa, T. Yamauchi, T. Kadowaki, Y. Takeuchi, H. Shibuya, Y. Gotoh, K. Matsumoto, S. Kato, Cytokines suppress adipogenesis and PPAR-gamma function through the TAK1/TAB1/NIK cascade, Nat. Cell Biol. 5 (2003) 224–230.

[16] M. Watanabe, J. Yanagisawa, H. Kitagawa, K. Takeyama, S. Ogawa, Y. Arao, M. Suzawa, Y. Kobayashi, T. Yano, H. Yoshikawa, Y. Masuhiro, S. Kato, A subfamily of RNA-binding DEAD-box proteins acts as an estrogen receptor alpha coactivator through the N-terminal activation domain (AF-1) with an RNA coactivator, SRA, EMBO J. 20 (2001) 1341–1352.

[17] J.G. Menke, K.L. Macnaul, N.S. Hayes, J. Baffic, Y.S. Chao, A. Elbrecht, L.J. Kelly, M.H. Lam, A. Schmidt, S. Sahoo, J. Wang, S.D. Wright, P. Xin, G. Zhou, D.E. Moller, C.P. Sparrow, A novel liver X receptor agonist establishes species differences in the regulation of cholesterol 7alpha-hydroxylase (CYP7a), Endocrinology 143 (2002) 2548–2558.

[18] J. Torchia, D.W. Rose, J. Inostroza, Y. Kamei, S. Westin, C.K. Glass, M.G. Rosenfeld, The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function, Nature 387 (1997) 677–684.

[19] N.J. McKenna, B.W. O'Malley, Combinatorial control of gene expression by nuclear receptors and coregulators, Cell 108 (2002) 465–474.

[20] B. Belandia, M.G. Parker, Nuclear receptors: a rendezvous for chromatin remodeling factors, Cell 114 (2003) 277–280.

[21] J. Huuskonen, P.E. Fielding, C.J. Fielding, Role of p160 coactivator complex in the activation of liver X receptor, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 703–708.

[22] F. Picard, M. Gehin, J. Annicotte, S. Rocchi, M.F. Champy, B.W. O'Malley, P. Chambon, J. Auwerx, SRC-1 and TIF2 control energy balance between white and brown adipose tissues, Cell 111 (2002) 931–941.

[23] M. Ito, C.X. Yuan, S. Malik, W. Gu, J.D. Fondell, S. Yamamura, Z.Y. Fu, X. Zhang, J. Qin, R.G. Roeder, Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators, Mol. Cell 3 (1999) 361–370.

[24] J.C. Yoon, P. Puigserver, G. Chen, J. Donovan, Z. Wu, J. Rhee, G. Adelmant, J. Stafford, C.R. Kahn, D.K. Granner, C.B. Newgard, B.M. Spiegelman, Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1, Nature 413 (2001) 131–138.

[25] M. Monsalve, Z. Wu, G. Adelmant, P. Puigserver, M. Fan, B.M. Spiegelman, Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1, Mol. Cell 6 (2000) 307–316.

[26] P. Puigserver, G. Adelmant, Z. Wu, M. Fan, J. Xu, B. O'Malley, B.M. Spiegelman, Activation of PPARgamma coactivator-1 through transcription factor docking, Science 286 (1999) 1368–1371.

[27] A.E. Wallberg, S. Yamamura, S. Malik, B.M. Spiegelman, R.G. Roeder, Coordination of p300-mediated chromatin remodeling and TRAP/mediator function through coactivator PGC-1alpha, Mol. Cell 12 (2003) 1137–1149.

[28] Z. Herceg, W. Hulla, D. Gell, C. Cuenin, M. Lleonart, S. Jackson, Z.Q. Wang, Disruption of Trrap causes early embryonic lethality and defects in cell cycle progression, Nat. Genet. 29 (2001) 206–211.