THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 1, pp. 20–26, January 6, 2006

An hGCN5/TRRAP Histone Acetyltransferase Complex Co-activates BRCA1 Transactivation Function through Histone Modification*

Received for publication, September 15, 2005 Published, JBC Papers in Press, October 31, 2005, DOI 10.1074/jbc.M510157200

Hajime Oishi[‡§], Hirochika Kitagawa[‡], Osamu Wada[‡§], Shinichiro Takezawa[‡¶], Làszlò Tora[‖], Madoka Kouzu-Fujita[‡§], Ichiro Takada[‡¶], Tetsu Yano[§], Junn Yanagisawa[‡], and Shigeaki Kato[‡¶1]

From the [‡]Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-0032, Japan, [§]Department of Obstetrics and Gynecology, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan, [‖]Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, F-67404 Illkirch Cedex, CU de Strasbourg, France, and [¶]ERATO, Japan Science and Technology, Honcho 4-1-8, Kawaguchi, Saitama 332-0012, Japan

It is well established that genetic mutations that impair BRCA1 function predispose women to early onset of breast and ovarian cancer. However, the co-regulatory factors that support normal BRCA1 functions remain to be identified. Using a biochemical approach to search for such co-regulatory factors, we identified hGCN5, TRRAP, and hMSH2/6 as BRCA1-interacting proteins. Genetic mutations in the C-terminal transactivation domain of BRCA1, as found in breast cancer patients (Chapman, M. S., and Verma, I. M. (1996) *Nature* 382, 678–679), caused the loss of phys- ical interaction between BRCA1 and TRRAP and signific- reduced the co-activation of BRCA1 transactivation f- hGCN5/TRRAP. The reported transcriptional squ- BRCA1 and estrogen receptor α (Fan, S., Wan- Meng, Q., Erdos, M. R., Pestell, R. G., Yu- berg, I. D., and Rosen, E. M. (1999) rescued by the overexpression transferase hGCN5 activ- regulator complex func- tion and DNA repair. TRRAP-containing comp- formed a complex with hMS- class of hGCN5/TRRAP-cont- containing)-type histone ace- hPCAF, and hSTAGA) (Yanagisa- Wada, O., Ogawa, S., Nakagomi, M.- sawa, H., McMahon, S. B., Cole, M. D- Kato, S. (2002) *Mol. Cell* 9, 553–562). -es, the isolated complex harbored a previously -bination of components including hMSH2 and hMSH- components of the BRCA1 genome surveillance repair complex (BASC). Thus, our results suggested that the multiple BRCA1 functions require a novel hGCN5/TRRAP histone acetyltransferase complex subclass.

Germ line mutations in BRCA1 are known to predispose women to the early onset of breast and ovarian cancer (1, 2). The 1863-amino acid BRCA1 protein is unique in that it harbors an N-terminal RING domain and two tandem copies of BRCT[2] at its C-terminal end (8). The major function of BRCA1 is thought to be as a tumor suppressor via DNA repair and transcriptional co- presumably involving chromatin remodeling and histon- (10). The transcription factor function of BRCA1 other classes of sequence-spe- cific regulato- autonomous transactivation domain- -nce of the BRCT domain is fu- -er of BRCA1 mutations T domain with resultant despite the pivotal role -uppression, little is -mplexes that sup-

-atin remodel- -olving chromo- -A-interacting factors -t of them appear to exhibit -n remodeling functions (10, 15). specific regulators and co-regulators, -modification are thought to be tightly cou- acetylation appears to often initiate gene regula- -ed by further histone modification, including methyl- phosphorylation (10, 16, 17). However, the molecular -anisms by which these processes of histone modification are con- -rolled remain largely unknown with many histone modification com- plexes still to be identified. Although it is known that several histone acetyltransferases (HATs) and HAT-containing complexes co-regulate sequence-specific regulators (5, 18–21), it is unclear whether each sequence-specific regulator requires a cognate HAT complex (or com- plexes) or can share common HAT complexes with other sequence- specific regulator classes.

It was reported recently that the transactivation function of BRCA1 was squelched by the transactivation of ERα (4, 22), considered to be a critical regulator of estrogen-dependent breast cancer. ERα is a member of the nuclear receptor gene superfamily, acting as a hormone-depend- ent transcription factor, and is known to require a number of chromatin remodeling and histone modification complexes. As two HAT co-acti-

* This work was supported in part by a grant-in-aid for priority areas from the Ministry of Education, Science, Sports, and Culture of Japan (to S. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom correspondence should be addressed: Inst. of Molecular and Cellular Biosciences, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-0032, Japan. Tel.: 81-3-5841-8478; Fax: 81-3-5841-8477; E-mail: uskato@mail.ecc.u-tokyo.ac.jp.

[2] The abbreviations used are: BRCT, BRCA1 C-terminal domain; GCN5, general control non-derepressible 5; TRRAP, transformation/transcription domain-associated protein; HAT, histone acetyltransferase; ERα, estrogen receptor α; LBD, ligand binding domain; GST, glutathione *S*-transferase; ID, interaction domain; ERE, estrogen-responsive element; TFTC, TATA-binding protein (TBP)-free TBP-associated factor (TAF)-containing; RNAi, RNA interference; MMS, methylmethane sulfonate; BASC, BRCA1 genome surveillance repair complex; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

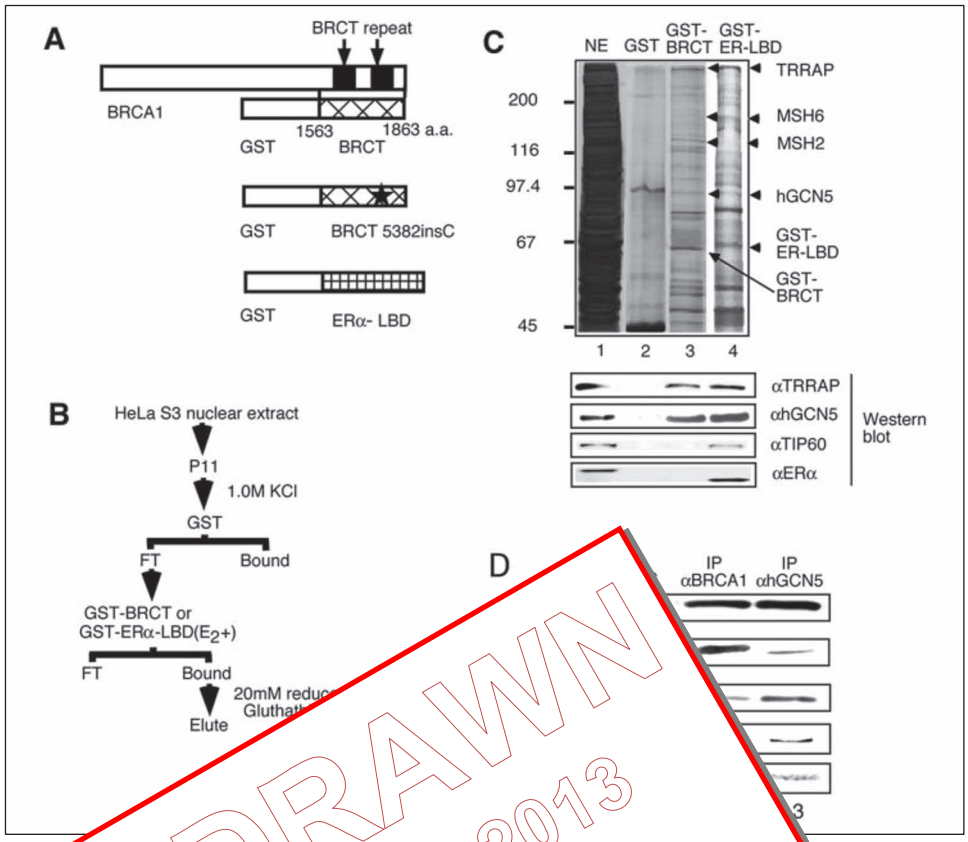

FIGURE 1. **Biochemical purification of a BRCT-interacting hGCN5/TRRAP complex.** *A*, schematic illustration of BRCA1, GST-fused BRCT proteins, and GST-fused ERα-LBD. *B*, purification schema for BRCT or estrogen-bound ERα-interacting proteins. Elution fractions from P11 columns were passed through immobilized GST columns and loaded onto immobilized GST-BRCT or GST-ERα (LBD) in the presence of estrogen (E_2) (10^{-6} M). *C*, identification of BRCT-interacting proteins. BRCT-interacting proteins contained TRRAP, hGCN5, MSH2, and MSH6 in common with liganded ERα fractions (*arrows*). Total HeLa S3 nuclear extracts (*lane 1*), fractions bound by the GST columns (*lane 2*), fractions eluted from the GST-BRCT columns (*lane 3*), and fractions eluted from liganded GST-ERα (LBD) fractions (*lane 4*) were subjected to SDS-PAGE followed by silver staining. Proteins from GST-BRCT columns were examined by MALDI-TOF MS with identified proteins indicated at the *right* side of the panel. The identity of the components in the *lower panel* was further confirmed by Western blotting. *D*, endogenous BRCA1-associated hGCN5/TRRAP complexes were co-immunoprecipitated with anti-BRCA1 and anti-hGCN5 antibodies. Whole cell extracts from subconfluent proliferating MCF7 cells were subjected to immunoprecipitation. RAD50, a major component of the BRCA1-associated repair complex (BASC), co-immunoprecipitated with endogenous BRCA1 and hGCN5. *IB*, immunoblot; *IP*, immunoprecipitation; *FT*, flow-through.

vator complexes have already been repo… …ned from the induced transactivation function … …t 10% fetal bovine BRCA1 and ERα share a co… this issue, we searched … …the purification of BRCT-in- hERα and BRCT and bio… …ear extracts from 10^{10} MCF7 stable plex (23, 24). Both TRRA… …d using standard procedures (21). The transactivation function, and … …ssed through a glutathione *S*-transferase (GST) scriptional squelching observed … …ound to a GST-BRCT column. After washing with 10 cal purification of the TRRAP/… …nes of washing buffer (20 mM Tris-HCl (pH 7.9), 150 mM ated with BRCA1 identified a nove… …2 mM EDTA, 0.05%, 0.5 mM phenylmethylsulfonyl fluoride, 0.05% Thus, our study suggested that a no… …onidet P-40, and 10% glycerol), complexes were eluted with 20 mM subclass is required for the multiple fu… reduced glutathione in 3 ml of elution buffer (50 mM Tris-HCl (pH 7.9), 150 mM KCl, 0.5 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.08% Nonidet P-40, and 10% glycerol). Bound proteins were then loaded onto 3-ml M2 anti-FLAG-agarose gels (Eastman Kodak Co.) and then eluted by incubation with 10 ml of FLAG peptide at 0.2 mg/ml (Sigma) in binding buffer. For fractionation on glycerol density gradients, 1-ml eluants were layered onto the top of 13-ml linear 100–10% glycerol gradients and centrifuged for 14 h at 40,000 rpm in an SW40 rotor (Beckman). All the procedures were done at 4 °C. Protein standards used were β-globulin (158 kDa) and thyroglobulin (667 kDa).

MATERIALS AND METHODS

Expression Vectors—Full-length BRCA1 … …5 cDNA were inserted into pcDNA3 vectors with FLAG … …s/MYC epitope tags (Invitrogen). The BRCT domain was cloned into pGEX4T-1 (Amersham Biosciences). BRCA1 and hGCN5 point mutants were generated using the QuikChange mutagenesis kit (Stratagene). Adenoviral vectors were constructed using the Adeno-X expression system (Clontech) according to the manufacturer's instructions. Sequences for short hairpin RNAi were inserted into the H1 RNA promoter vector (25, 26). Targeted RNAi sequences used were TRRAP-RNAi (5′-CCAGGGGTGCCAGGCGGGGTCCATGCGG-3′), hGCN5 (5′-GCCGGGGTCGGGGCCTGGGAAGGTTCCG-3′), and hMSH2 (5′-CTCTCCAACTGCAGCGTCTCCTTCGGCT-3′). A scrambled sequence (5′-ATCTCACCCATCACACGGAGCCGCTTGC-3′) was used as a negative control. Other vectors were as described previously (16, 21, 27).

Cell Culture—Human MCF7 breast cancer cells were transformed to stably express hGCN5 as described previously (21) and maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum con-

Immunoprecipitation—For immunoprecipitation of endogenous BRCA1 or hGCN5, subconfluent 5×10^7 MCF7 cells were harvested, washed twice with ice-cold phosphate-buffered saline, and resuspended in 5 ml of ice-cold lysis buffer (50 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 1 mM EDTA, and 1.0% Triton X-100). Whole cell extracts were then immunoprecipitated using 10 μg of each antibody (anti-BRCA1 (BRCA1–17F8, Gene Tex), hGCN5 (H-75, Santa Cruz Biotechnology), or non-immune rabbit IgG (negative control)). The precipitated proteins were collected using protein G-Sepharose beads (Amersham Bio-

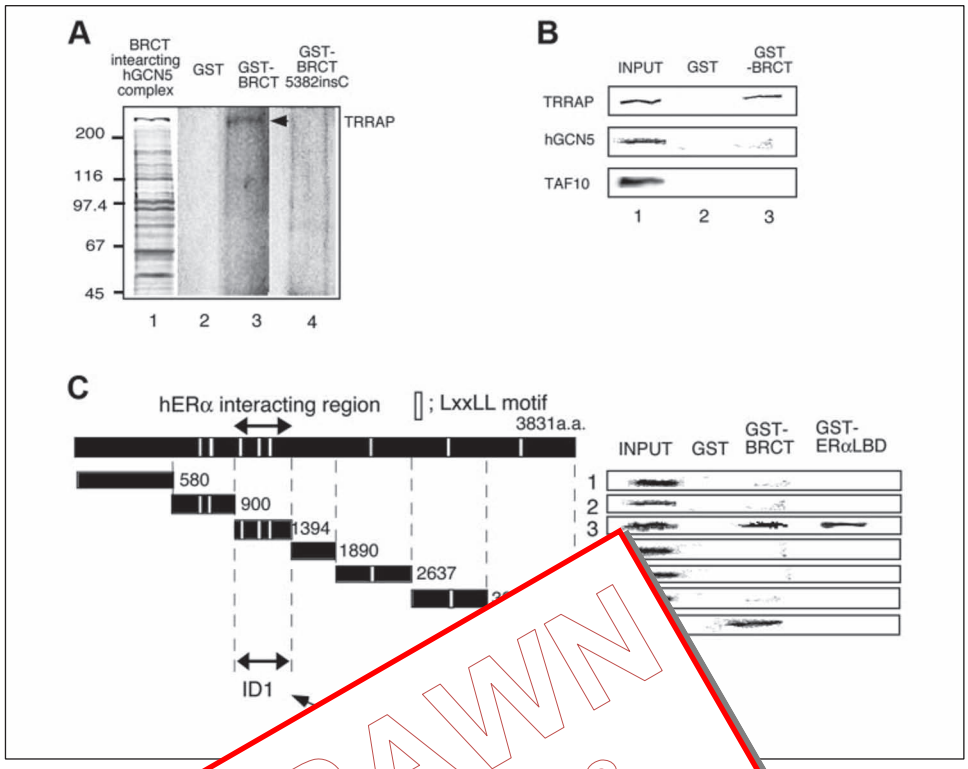

FIGURE 2. **BRCA1 associates with an hGCN5/TRRAP complex in MCF7 cells.** *A*, far Western blotting of BRCT-interacting hGCN5 complex components and BRCT. BRCT-interacting hGCN5 complexes (*lane 1*) were analyzed by far Western blotting and visualized by silver staining (10% of input shown). Each probe (*lanes 2–4*) was detected using an anti-GST-specific antibody specific for GST-fused BRCT (16). At the *right* side of the panel, an *arrow* shows TRRAP as a direct interactant; this was confirmed by Western blotting (data not shown). *B*, physical interaction between the complex components and BRCT was confirmed by GST pull-down assay. TRRAP binding was determined by SDS-PAGE autoradiography. *C*, mapping of the BRCT- or ERα-interacting region of TRRAP by GST pull-down assay using TRRAP fragments and GST-BRCT or GST-ERα-LBD. BRCT-interacting domains were designated as ID1 and ID2 as shown at the *bottom* of the panel. TRRAP ID1 interacts with both BRCT and ERα-LBD. *a.a., amino acids.*

sciences), washed, eluted in sample buffer, and subi
blotting analysis (28).

GST Pull-down Assay—GST fusion pro
erichia coli and bound to glutathion
Biosciences). *In vitro* translated
lation using the T7 pro
labeled using [^{35}S]meth
translation was carried ou
lysate system (Promega). T
incubated with beads for 1 h a
washing the beads with NET-N
mM Tris-HCl (pH 7.5), 0.5% Noni
fluoride, and 1 mM dithiothreitol). B
sample buffer, separated by SDS-PAG
raphy. For all GST pull-down assays, t
protein amount used in the assays.

Antibodies—For Western blotting, a
gainst BRCA1
(BRCA1–17F8, Gene Tex), TRRAP (H-300, S
ruz Biotechnology),
hGCN5 (C-20, Santa Cruz Biotechnology), hMSH2 (N-20, Santa Cruz Biotechnology), hMSH6 (N-20, Santa Cruz Biotechnology), RAD50 (13B3, Gene Tex), and TIP60 (K-17, Santa Cruz Biotechnology) were used. For the chromatin immunoprecipitation assay, we used antibodies against BRCA1 (MS-BRC14-UP50, Upstate Biotechnology, Inc.), TRRAP (T-17, Santa Cruz Biotechnology), hGCN5 (H-75, Santa Cruz Biotechnology), and acetyl-H3 (Upstate Biotechnology, Inc.).

Chromatin Immunoprecipitation Assay—Preparation of soluble MCF7 chromatin for PCR amplification was performed as described previously (21). Specific primer pairs for the p21 promoter region used were 5′-TCCAGCGCACCAACGC-3′ and 5′-AGCTGCTCACACCTCAG-3′. Subconfluent MCF7 cells were infected with adenoviral vectors (Adeno-LacZ, V; Adeno-BRCA1, BRCA1; and Adeno-BRCA1 5382insC, BRCTmt) for 24 h at a multiplicity of infection of 20 to ensure expression in more than 90% of cells. Cells were then treated with 0.01%

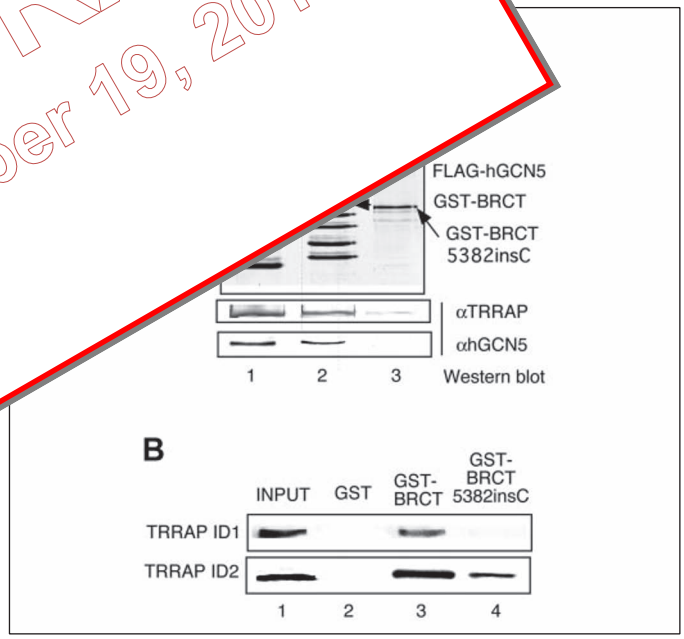

FIGURE 3. **The BRCT mutation BRCT insC abolished interaction with BRCT-interacting TRRAP/hGCN5 complexes.** *A*, hGCN5 complexes were applied to immobilized GST-BRCT beads. *Lane 1* shows hGCN5 complex (5% of input) immunoprecipitated from FLAG-hGCN5 stable transformant MCF7 cells by anti-FLAG antibody. Amounts of GST-fused proteins were equalized. Bound proteins were eluted and subjected to silver staining. *B*, BRCT mutation abolished interaction with TRRAP. The binding of wild-type or clinical mutant BRCT to TRRAP ID1 was analyzed by GST pull-down assay.

methylmethane sulfonate (MMS) for 60 min, washed with phosphate-buffered saline, and incubated for 60 min with fresh medium.

Northern Blot Analysis—Total cellular RNA was isolated from the indicated cells using ISOGEN reagent (Wako Co.), and 20 mg RNA were used for Northern blot analysis with digoxigenin-dUTP-labeled cDNA probes. Probes were synthesized using a PCR digoxigenin probe

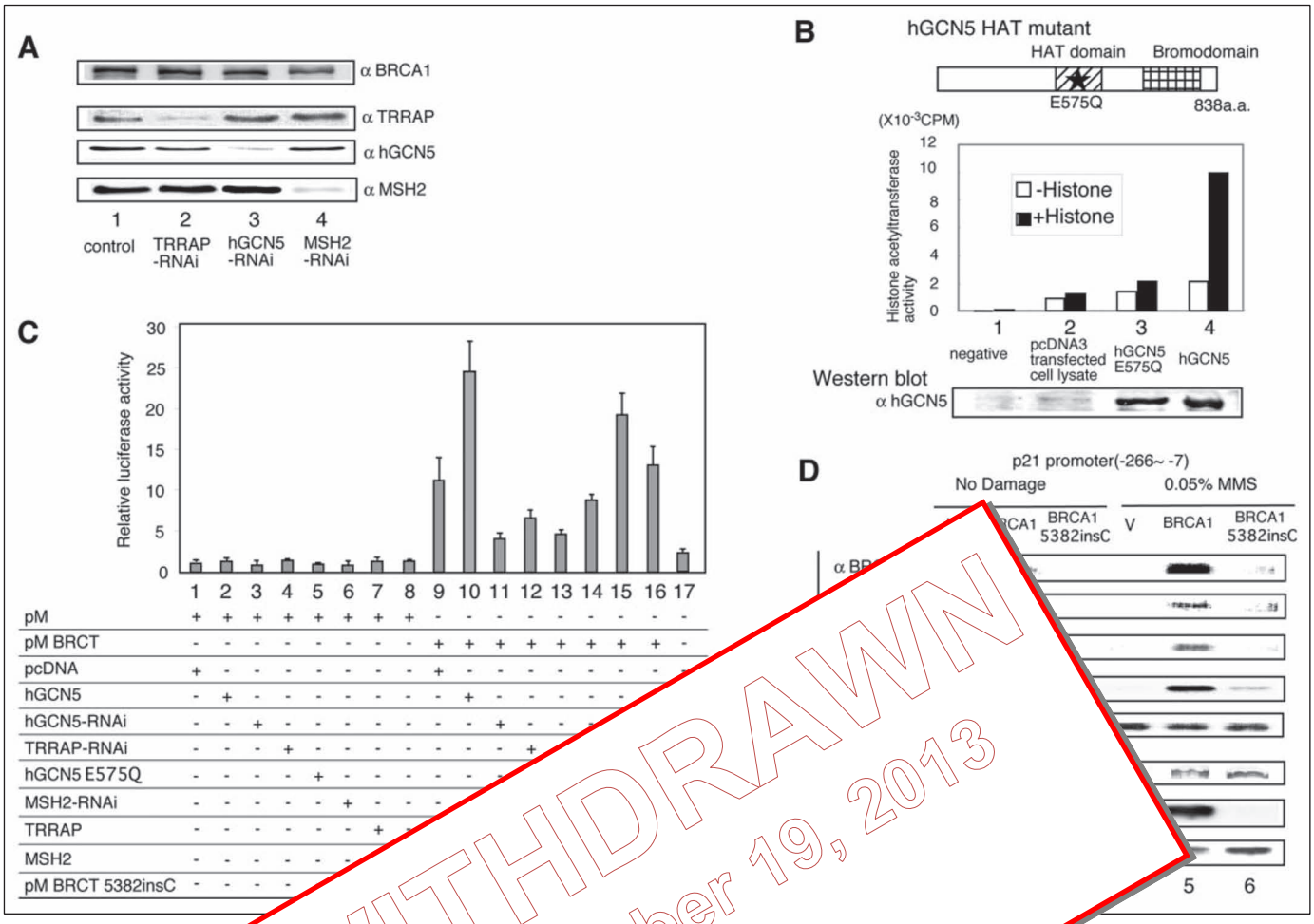

FIGURE 4. **A BRCT-interacting** …oteins by RNAi. The indicated RNAi vectors were transfected into … expression levels were unchanged. For control (*lane 1*), scramble seque… …dation (E575Q) corresponds to the yeast GCN5 HAT-abolished mutant (34). Subc… …lysates were incubated with either free histones (*closed bars*) or bovine serum albur… …nding assay (21, 28). *Lane 1* shows no cell lysate added. *C*, co-activation of BRCT by hGCN5 a… …using a luciferase reporter construct containing the GAL4 DNA binding site (17M8) showed spe… …12-well dishes were transfected overnight with the indicated expression vectors and reporter plasmid… …gen). Total amount of DNA in each transfection was adjusted to 1.0 μg by supplementing with empty vectors. L… …tors (*lane 1*). Results represent the average of at least three independent experiments; *error bars* indicate standard … …promoter along with BRCA1. MCF7 cells were infected with Adeno-LacZ (V), Adeno-BRCA1 (BRCA1), or Adeno-BRCA1 538… …min. Soluble chromatin fractions were applied to chromatin immunoprecipitation analysis. Induction of the p21 gene by DNA da… …sis. *a.a.*, amino acids; *ChIP*, chromatin immunoprecipitation.

synthesis kit. The p21 cDNA probe was am… …c primers (5′-ATGTCAGAACCGGCTGGG-3′ and …GAAGTAGCT-GGCAT-3′) (28).

Cell Survival Assay—HCC1937 cells plated on 60-mm dishes at 40% confluency were transfected with pcDNA expression vectors (0.8 μg) and pSh-RNAi vectors (0.2 μg) using Effectene™ (Qiagen). Total DNA amounts were adjusted by supplementing up to 1.0 μg with empty pcDNA vector or scrambled sequence-inserted RNAi vector. After 24 h, transfected cells were treated with medium containing 0.1% MMS for 50 min, washed with phosphate-buffered saline, and maintained for 8 days in fresh medium. Surviving cells were then counted (12).

RESULTS

Biochemical Purification of hGCN5 and TRRAP as BRCA1 Interactants—To identify the co-regulators responsible for BRCT function, we biochemically purified protein complexes that interacted with BRCT. Recombinant BRCT domains (residues 1563–1863) fused to GST were immobilized to glutathione-Sepharose beads and used as a purification probe (Fig. 1, *A* and *B*). A number of BRCT-associating proteins were purified from HeLa S3 nuclear extracts with a 400-kDa protein identified as TRRAP and a 95-kDa protein as hGCN5 by MALDI-TOF MS (Fig. 1*C*). The HAT hGCN5 and the Myc-interacting protein TRRAP (5) are known to be present in several subtypes of TFTC/STAGA complex in combination with other components (6, 7).

Notably hMSH6 and hMSH2 were also identified as BRCT interactants. As hMSH2 and hMSH6 are thought to be major components of the BRCA1 genome surveillance repair complex (BASC) (29, 30) along with other DNA repair response proteins, it was possible that BASC was trapped to the BRCT domain along with the hGCN5/TRRAP complex. Although a TFTC-like complex has been purified previously from HeLa cell nuclear extracts and shown to serve as a ligand-dependent co-activator complex for ERα *in vitro* (21), ERα was undetectable in the BRCT interactants by our purification procedure (Fig. 1*C*). This suggested that we had isolated a novel hGCN5/TRRAP complex subclass that interacted with BRCT but not with ERα. Also as reported previously (31, 32), although TRRAP and hGCN5 were clearly detected by Western blot

analysis, TIP60, a component of another TRRAP-containing HAT complex subtype involved in DNA repair, interacted with ERα but not with BRCT (Fig. 1*C*). Association of BRCA1 with TRRAP, hGCN5, and MSH2 *in vivo* was confirmed by co-immunoprecipitation (Fig. 1*D*), and RAD50, a BASC component, also co-precipitated with BRCA1 as expected.

TRRAP Was a Direct Interactant of BRCT—We then attempted to identify BRCT interactants in the hGCN5/TRRAP-containing complex by far Western blot analysis. Of the purified interactants bound to GST-BRCT probes (Fig. 1*C*), we observed significant binding between TRRAP and BRCT (Fig. 2*A*). Direct and clear interaction of BRCT with TRRAP but not with hGCN5 was confirmed *in vitro* using a GST pull-down assay (Fig. 2*B*). This assay was repeated using TRRAP deletion mutants to map the interacting regions (Fig. 2*C*) and identified two domains in the N- and C-terminal regions, designated as ID1 and ID2, that appeared to serve as the direct interface for BRCT (Fig. 2*C*).

A BRCA1 Mutation Found in Breast Cancer Patients Abrogated the Physical Association with TRRAP—The L*XX*LL motif is well known to serve as a direct interface for liganded nuclear receptors via the C-terminal helix 12, and indeed three L*XX*LL motifs were found to be responsible for stable association with liganded hERα (see Fig. 2*C*) (21). Interestingly ID1, but not ID2, overlapped with the hERα-interacting region (see Fig. 2*C*). Thus, the mode of the BRCT interaction with TRRAP appears to be distinct from that of hERα. The physiological relevance of the observed interaction between BRCT and the hGCN5/TRRAP co
plex was then verified using a BRCA1 mutant (5382insC) f
breast cancer cell line HCC1937 that exhibits reduced t
function (33). When this mutant BRCA1 was use
probe, hGCN5/TRRAP complex component
3*A*). Indeed a GST pull-down assay
domain exhibited only poor inter
3*B*, *lower panel*). Moreover
in the complete loss of
Far Western blotting also
TRRAP (Fig. 2*A*). Therefo
cancer patients may exhibit d
of reduced association with TR

*Co-activation of BRCA1 Tr
through Histone Acetylation*—To
hGCN5 HAT complex served as a co
co-activator functions of the purified
transient expression assay in MCF7 c
BRCT
domain linked to the yeast GAL4 DNA-bi
lear potenti-
ation of BRCT function by hGCN5 overexp
was observed (see
Fig. 4*C*, *lane 10*), whereas the RNAi-mediated reduction of endogenous hGCN5 levels (see Fig. 4*A*, *lane 3*) (25, 26) resulted in reduced BRCT transactivation function (Fig. 4*C*, *lane 11*). Likewise both TRRAP and MSH2 alone appeared to co-activate BRCT at least to some extent (Fig. 4*C*). Based on the findings that HAT activity is essential for HAT complex co-activator-mediated transactivation of sequence-specific regulators, we tested an hGCN5 point mutant known to abolish HAT activity through the replacement of a glutamic acid (Glu) with a glutamine (Gln) at position 575 (E575Q) (34). Results of the HAT assay confirmed the loss of HAT activity of this mutant even though the mutant clearly overexpressed (Fig. 4*C*, compare *lanes 3* and *4* with wild-type hGCN5). The E575Q mutation clearly abrogated the hGCN5-mediated co-activator activity on BRCT (Fig. 4*C*, compare *lane 10* with *lane 13*), which suggested that the GCN5 HAT complex was a critical component of the co-activator complex involved in BRCA1 transactivation function.

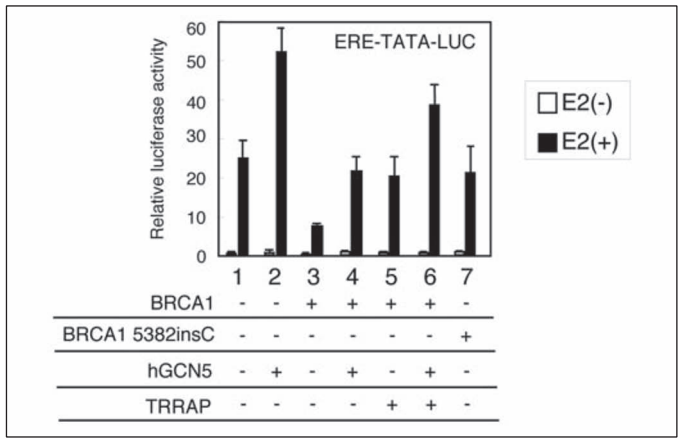

FIGURE 5. **Abrogation of transcriptional interference between BRCA1 and liganded ERα by hGCN5 and TRRAP.** Transcriptional activity in the presence of the indicated factors was examined using a luciferase reporter construct containing the ERE-TATA. Subconfluent proliferating MCF7 cells in 12-well dishes were transfected overnight with the indicated expression vectors and
er plasmid (ERE-TATA-luc) (50 ng for each lane) using Effectene (Qiagen). To
DNA in each transfection was adjusted to 0.3 μg by supplementing wi
uciferase activity is expressed relative to negative control (*lane 1*
verage of at least three independent experiments; *error b
E_2, estrogen.

T
ere indeed recruited to
ecific activator at target
unoprecipitation assay
CA1 target (35). As
exogenous BRCA1
1 promoter was
MMS-induced
hGCN5 and
hyperacetylation
hGCN5 HAT recruited

ession Abrogated Transcriptional
—We then examined whether hGCN5
escue the reported transcriptional squelching
BRCA1. Estrogen-induced transactivation of hERα
ensus estrogen-responsive elements (EREs) was potenti-
hGCN5 (Fig. 5, *lane 2*). In contrast, BRCA1 overexpression
elched the ligand-induced transactivation of hERα (Fig. 5, compare *lane 1* with *lane 3*) as previously reported. Interestingly the BRCA1 5382insC mutant failed to squelch hERα transactivation (Fig. 5, *lane 7*), suggesting the possibility of common co-activator(s) between hERα and BRCA1. This hypothesis was supported by the finding that hGCN5 and TRRAP were both able to abrogate the transcriptional squelching of hERα by BRCA1 (Fig, 5, *lanes 4* and *5*) and that an additive effect was observed when both factors were used together (Fig. 5, *lane 7*). These findings suggested that hGCN5 HAT and TRRAP are common co-activators for both hERα and BRCA1.

Biochemical Identification of a Novel hGCN5/TRRAP HAT Complex—To identify the components of the BRCT-interacting hGCN5/TRRAP complex and to test whether the hGCN5/TRRAP complex was trapped to BRCA1, we established an MCF7 stable transformant cell line expressing FLAG-tagged hGCN5. Complexes from this cell line were purified by two-step column chromatography followed by glycerol density gradient sedimentation (Fig. 6*A*) (16). Unlike TFTC-type co-activator complexes, the proteins SAP130, TAF5, and TAF6 appeared to be absent (18, 36), whereas TAF10 was present (37, 38). Notably hMSH2 and hMSH6 were also identified by MALDI-TOF MS and were further confirmed by Western blot analysis (Fig. 6, *B* and *C*). However,

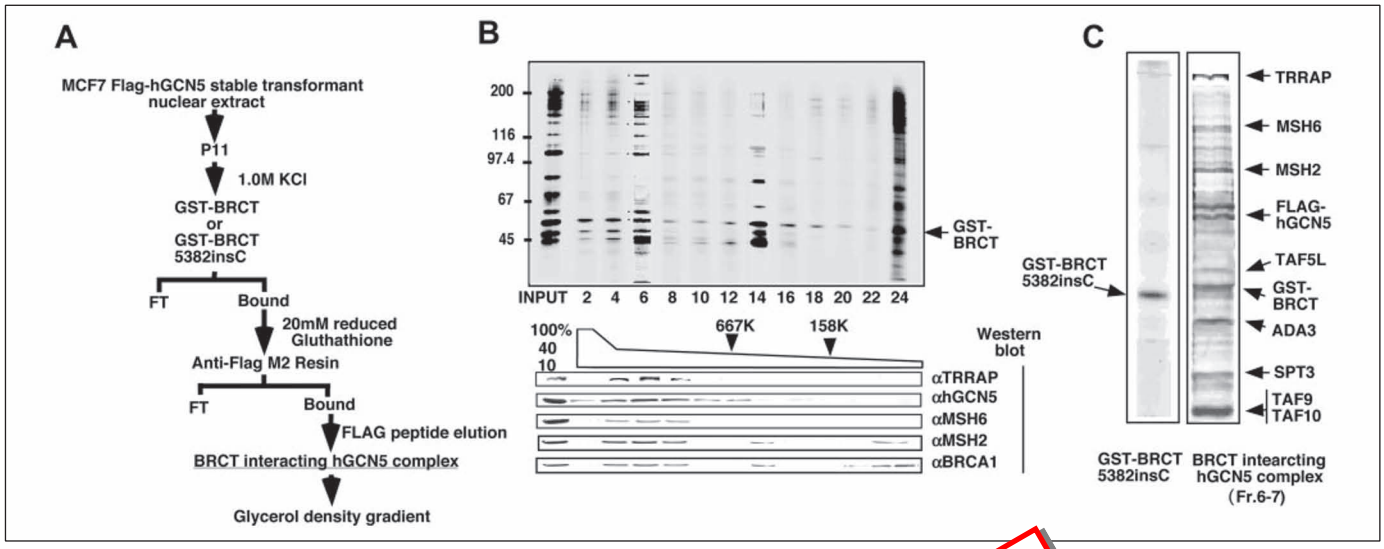

FIGURE 6. **Identification of an hGCN5/TRRAP complex associating with BRCA1.** *A*, purification schema for BRCT-interacting ... plexes and hGCN5 complexes from MCF7 stable transformants. *B*, fractionation of purified complexes on glycerol density gradients. BRCT-interacting hGCN5 com... ated on a 10–40% glycerol gradient by centrifugation. *INPUT* shows GST-BRCT-bound and anti-FLAG-purified material. *INPUT* and fractions from the *top* ... by SDS-PAGE and visualized by silver staining. The positions of the marker proteins of known molecular masses are shown as *arrows*. The *lower p*... alysis of each fraction using specific antibodies against TRRAP, hGCN5, MSH6, MSH2, and BRCA1. Co-fractionation of TRRAP, hGCN5, MSH6, M... C, purified complexes subjected to SDS-PAGE and silver staining. Purified fractions (*B*, fractions 6 and 7) were separated by SDS-PAGE. GS... Components were identified by mass spectrometry and confirmed by Western blotting with specific antibodies (data not shown).

other BASC components were not detected, suggesting that the purif... hGCN5/TRRAP complex(es) did not associate with BASC s... hMSH2 and hMSH6 formed part of the hGCN5/TRRAP...

hGCN5 HAT Activity Was Required for hGCN5-i... tion of BRCA1 DNA Repair Function—Fin... BRCA1-associated hGCN5/TRRAP co... mediated DNA repair, we measure... age in HCC1937 cells with ... TRRAP components. ... enhanced cell survival, th... cell survival (Fig. 7, compar... potentiated the DNA repair ... the hGCN5 HAT mutant (hG... nant negative hGCN5 (see *lane 1*... identified hGCN5/TRRAP comple... mediated DNA repair via its HAT a...

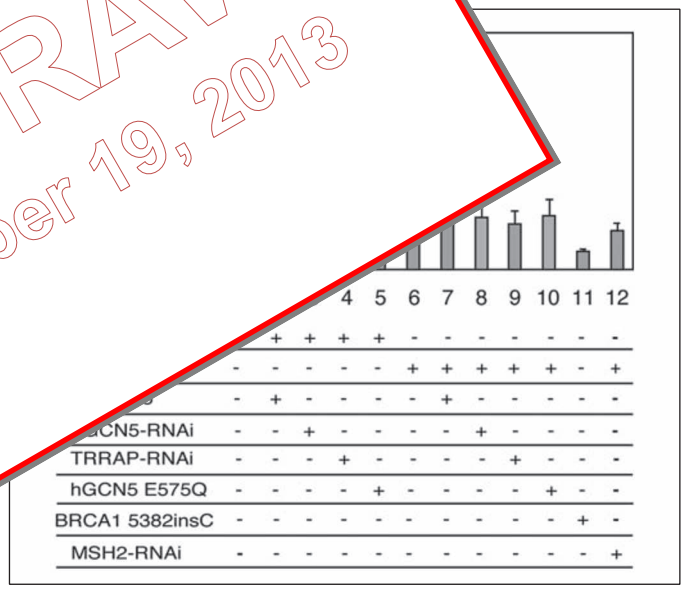

FIGURE 7. **Association between the BRCT domain and hGCN5 complex is required for BRCA1-mediated cell survival after DNA damage.** Parallel cultures of HCC1937 cells transfected with the indicated vectors were treated with 0.1% MMS for 50 min. Cells surviving DNA damage were counted. Although BRCA1-transfected cells restored MMS resistance, loss of BRCT-interacting hGCN5 complex components showed reduced resistance (12).

DISCUSSION

Core Components of the hERα-interacti... plex Were Identified Along with hM... RCT Domain Interactants—BRCA1 has been well establish... a tumor suppressor of breast and ovarian cancers through DNA repair control and gene regulation. Although several factors and complexes, including BASC, have been identified as being involved in the BRCA1-mediated DNA repair, it remains unclear how these factors/complexes support BRCA1 functions at specific stages of the DNA repair process. Similarly gene regulation by BRCA1 also appears to require several factors/complexes, but the functional significance of these factors/complexes in BRCA1 function is largely unknown. To address these issues, we biochemically purified and identified protein interactants for BRCT, the C-terminal domain thought to support the transactivation function of BRCA1. Our study identified TRRAP, hGCN5, hMSH2, and hMSH6 as BRCT interactants, and their association with BRCA1 was confirmed using both cellular and *in vitro* approaches. Importantly hGCN5 was found to co-activate BRCA1 function; the HAT activity of hGCN5 apparently was indispensable for BRCT co-activation. In support of these findings, the histone hyperacetylation detected using BRCA1 could be potentiated by hGCN5 overexpression, whereas an hGCN5 mutant lacking HAT activity was unable to co-activate BRCT transactivation function. As hGCN5 is well known to associate with TRRAP to form a transcriptional co-activator complex (6, 7), it was likely that hGCN5 co-activated BRCT function with TRRAP as part of a complex. However, none of the subclasses of hGCN5/TRRAP-containing TFTC-like complexes reported so far also contain hMSH2 and hMSH6.

hGCN5 HAT Co-activated BRCA1 Function through Histone Modification—Biochemical purification of BRCA1-interacting complexes from MCF7 cells stably expressing hGCN5 identified a TFTC/

STAGA complex subclass that appeared to support the transactivation function of BRCA1 via the BRCT domain. The identified hGCN5/TRRAP complex was distinct from other TFTC/STAGA complex subclasses (6, 39) as it contained hMSH2 and hMSH6, major functional components of BASC (30). The presence of these DNA repair-related factors in the hGCN5/TRRAP complex suggested that BRCA1-mediated DNA repair required histone modification via the HAT activity of hGCN5 presumably as part of the TRRAP-containing complex. Given the finding that the physical and functional association between BRCA1 and TRRAP could be disrupted by using BRCT mutants observed in breast cancer patients, it is likely that the hGCN5/TRRAP complex identified in our study plays a pivotal role, via its histone acetylation activity, in the diverse functions of BRCA1, including DNA repair and gene regulation.

Although hMSH2 and hMSH6 were present in the biochemically purified hGCN5/TRRAP complex, other BASC components were not. Given that RAD50, a major BASC component (29, 30), was co-immunoprecipitated with BRCA1 in agreement with previous reports (12), the hGCN5/TRRAP complex may form part of a larger complex with BASC to fully support BRCA1 functions. As BRCT mutations found in breast cancer patients prevented interaction with the hGCN5/TRRAP complex, it is possible that the hGCN5/TRRAP complex is recruited first to allow histone modification as part of the transcriptional process involving hGCN5-mediated histone acetylation and perhaps allowing the recognition of damaged DNA. BASC may then associate with BRCA1 bound to the modified chromosome. To better understan
molecular basis of BRCA1 function, it would be interesting
gate the cooperative functions between the two compl
both transcriptional control and DNA repai
modification.

Multiple Nuclear Complexes Sup
become clear that DNA-bindin
number of nuclear comple
complexes associate wit
manner. As BRCA1 harbo
domain, it is likely that BR
ulator complexes in a sequenti
unlike other DNA-binding facto
a number of factors/complexes i

*Acknowledgments—We thank Dr. I. Da
providing antibodies and Dr. H. Nagasa
thank Y. Mezaki, Dr. F. Ohtake, Dr. M. Kim
Fujiki, A. Baba, Y. Sasaki, and Dr. K. Ishitani j
H. Higuchi for preparation of the manuscript.*

REFERENCES

1. Futreal, P. A., Liu, Q., Shattuck-Eidens, D., Cochran, C., Harshman, K., Tavtigian, S., Bennett, L. M., Haugen-Strano, A., Swensen, J., Miki, Y., Eddington, K., McClure, M., Frye, C., Weaver-Feldhaus, J., Ding, W., Gholami, Z., Söderkvist, P., Terry, L., Jhanwar, S., Berchuck, A., Iglehart, J. D., Marks, J., Ballinger, D. G., Barrett, J. C., Skolnick, M. H., Kamb, A., and Wiseman, R. (1994) *Science* **266,** 120–122
2. Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L. M., 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, P. K., Norris, F. H., Helvering, L., Morrison, P., Rosteck, P., Lai, M., Barrett, J. C., Lewis, C., Neuhausen, S., Cannon-Albright, L., Goldgar, D., Wiseman, R., Kamb, A., and Skolnick, M. H. (1994) *Science* **266,** 66–71
3. Chapman, M. S., and Verma, I. M. (1996) *Nature* **382,** 678–679
4. Fan, S., Wang, J., Yuan, R., Ma, Y., Meng, Q., Erdos, M. R., Pestell, R. G., Yuan, F., Auborn, K. J., Goldberg, I. D., and Rosen, E. M. (1999) *Science* **284,** 1354–1356
5. McMahon, S. B., Van Buskirk, H. A., Dugan, K. A., Copeland, T. D., and Cole, M. D. (1998) *Cell* **94,** 363–374
6. Brand, M., Moggs, J. G., Oulad-Abdelghani, M., Lejeune, F., Dilworth, F. J., Stevenin, J., Almouzni, G., and Tora, L. (2001) *EMBO J.* **20,** 3187–3196
7. Martinez, E., Palhan, V. B., Tjernberg, A., Lymar, E. S., Gamper, A. M., Kundu, T. K., Chait, B. T., and Roeder, R. G. (2001) *Mol. Cell. Biol.* **21,** 6782–6795
8. Scully, R., and Livingston, D. M. (2000) *Nature* **408,** 429–432
9. Venkitaraman, A. R. (2002) *Cell* **108,** 171–182
10. Narlikar, G. J., Fan, H. Y., and Kingston, R. E. (2002) *Cell* **108,** 475–487
11. Ludwig, T., Fisher, P., Ganesan, S., and Efstratiadis, A. (2001) *Genes Dev.* **15,** 1188–1193
12. Zhong, Q., Chen, C. F., Li, S., Chen, Y., Wang, C. C., Xiao, J., Chen, P. L., Sharp, Z. D., and Lee, W. H. (1999) *Science* **285,** 747–750
13. Monteiro, A. N., August, A., and Hanafusa, H. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 13595–13599
14. Cantor, S. B., Bell, D. W., Ganesan, S., Kass, E. M., Drapkin, R., Grossman, S., Wahrer, D. C., Sgroi, D. C., Lane, W. S., Haber, D. A., and Livingston, D. M. (2001) *Cell* **105,** 149–160
15. Ye, Q., Hu, Y. F., Zhong, H., Ny elmont, A. S., and Li, R. (2001) *J. Cell Biol.* **155,** 911–921
16. Kitagawa, H., Fujiki, R Y., Uematsu, Y., Matsui, D., Ogawa, S., Unno, K., Oku T., Ito, T., Ishimi, Y., Nagasawa, H., Matsumot) *Cell* **113,** 905–917
17. Pet **14,** R546–R551
18 L. (1999) *J. Biol. Chem.* **274,**
B. W. (1995) *Science* **270,**
nd Nakatani, Y. (1996)
S., Nakagomi, M., D., Tora, L., Taka-
G. (2001) *Proc. Natl.*
Gamble, M., Naar, A. M., Erdju- L. P. (1999) *Nature* **398,** 824–828
d Roeder, R. G. (2002) *Proc. Natl. Acad. Sci.*
ds, R., and Agami, R. (2002) *Science* **296,** 550–553
A. A., Bernstein, E., Hannon, G. J., and Conklin, D. S. (2002) –958
Takeyama, K., Matsumoto, T., Kitagawa, H., Yamamoto, Y., Nohara, K., na, C., Krust, A., Mimura, J., Chambon, P., Yanagisawa, J., Fujii-Kuriyama, Y., nd Kato, S. (2003) *Nature* **423,** 545–550
28. 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
29. Futaki, M., and Liu, J. M. (2001) *Trends Mol. Med.* **7,** 560–565
30. Wang, Y., Cortez, D., Yazdi, P., Neff, N., Elledge, S. J., and Qin, J. (2000) *Genes Dev.* **14,** 927–939
31. Ikura, T., Ogryzko, V. V., Grigoriev, M., Groisman, R., Wang, J., Horikoshi, M., Scully, R., Qin, J., and Nakatani, Y. (2000) *Cell* **102,** 463–473
32. Gaughan, L., Brady, M. E., Cook, S., Neal, D. E., and Robson, C. N. (2001) *J. Biol. Chem.* **276,** 46841–46848
33. Scully, R., Ganesan, S., Vlasakova, K., Chen, J., Socolovsky, M., and Livingston, D. M. (1999) *Mol. Cell* **4,** 1093–1099
34. Langer, M. R., Tanner, K. G., and Denu, J. M. (2001) *J. Biol. Chem.* **276,** 31321–31331
35. Somasundaram, K., Zhang, H., Zeng, Y. X., Houvras, Y., Peng, Y., Wu, G. S., Licht, J. D., Weber, B. L., and El-Deiry, W. S. (1997) *Nature* **389,** 187–190
36. Hahn, S. (1998) *Cell* **95,** 579–582
37. Wieczorek, E., Brand, M., Jacq, X., and Tora, L. (1998) *Nature* **393,** 187–191
38. Struhl, K., and Moqtaderi, Z. (1998) *Cell* **94,** 1–4
39. Rodriguez-Navarro, S., Fischer, T., Luo, M. J., Antunez, O., Brettschneider, S., Lechner, J., Perez-Ortin, J. E., Reed, R., and Hurt, E. (2004) *Cell* **116,** 75–86