Ubiquitination and Proteasomal Degradation of the BRCA1 Tumor Suppressor Is Regulated during Cell Cycle Progression

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The BRCA1 tumor suppressor and the BARD1 protein form a stable heterodimeric complex that can catalyze the formation of polyubiquitin chains. Expression of BRCA1 fluctuates in a cell cycle-dependent manner, such that low steady-state levels of BRCA1 gene products are found in resting cells and early G1 cycling cells and high levels in S and G2 phase cells. Although transcriptional activation of the BRCA1 gene can account for induction of BRCA1 expression at the G1/S transition, the mechanisms by which BRCA1 is down-regulated during cell cycle progression have not been addressed. Here we show that the steady-state levels of BRCA1 protein remain elevated throughout mitosis but begin to decline at the M/G1 transition. This decline in BRCA1 levels coincides with the appearance of proteasome-sensitive ubiquitin conjugates of BRCA1 at the onset of G1. Formation of these conjugates occurs throughout G1 and S, but not in cells arrested in prometaphase by nocodazole. The proteasome-sensitive ubiquitin conjugates of BRCA1 appear to be distinct from BRCA1 autoubiquitination products and are probably catalyzed by the action of other cellular E3 ligases. Interestingly, co-expression of BARD1 inhibits the formation of these conjugates, suggesting that BARD1 serves to stabilize BRCA1 expression in part by reducing proteasome-sensitive ubiquitination of BRCA1 polypeptides. In summary, these data indicate that the cell cycle-dependent pattern of BRCA1 expression is determined in part by ubiquitin-dependent proteasomal degradation.

Germline mutations of the BRCA1 gene are responsible for a substantial proportion of hereditary breast and ovarian cancers (1, 2). In this clinical setting, BRCA1 serves as a tumor suppressor that contributes to tumorigenesis through loss of function. The protein it encodes has been implicated in a number of biological processes, including the cellular response to DNA damage (3, 4). In particular, BRCA1 is required for several checkpoints that control cell cycle progression (5, 6) and inhibit mRNA processing (7, 8) after genotoxic stress, as well as for certain modes of DNA repair such as nucleotide excision repair (9, 10) and homology-directed repair of double-strand DNA breaks (11–13). As a key regulator of the DNA damage response, BRCA1 presumably promotes tumor suppression by preserving genomic stability. However, the molecular mechanisms by which it carries out these functions are not understood and, as a consequence, it is still unclear why inherited mutations of the BRCA1 gene predispose women to breast and ovarian cancer.

The BRCA1 polypeptide contains two recognizable amino acid motifs: a RING domain near the N terminus and two tandem copies of the BRCT domain at the C terminus (14). In vivo, BRCA1 exists as a heterodimer with BARD1, a distinct protein that harbors a similar array of RING and BRCT motifs (15). Since the phenotypes of mice null for either Brca1 or Bard1 are essentially indistinguishable, the functions of both proteins are likely to be mediated through the BRCA1/BARD1 heterodimer (16), and indeed BARD1 has already been implicated with BRCA1 in homology-directed repair of chromosomal breaks (17). BRCA1 and BARD1 associate by assembling a stable 4-helix bundle from the α helices that flank their respective RING domains (18), and together they form an enzymatic complex that can catalyze ubiquitin polymerization in vitro (19–24). This enzymatic activity implies that BRCA1/BARD1 functions as an E3 ligase that promotes ubiquitin modification of specific substrate proteins, and that these are likely to include important effectors of BRCA1-mediated tumor suppression (25, 26). Although definitive substrates of BRCA1/BARD1 have not yet been identified, autoubiquitination of the BRCA1 subunit is observed during in vitro reactions catalyzed by BRCA1/BARD1 (22). In vitro, BRCA1/BARD1 directs the formation of ubiquitin polymers through an unconventional isopeptide linkage involving lysine residue K6 of ubiquitin (27, 28). These K6-linked polyubiquitin chains are distinct from the more common K48-linked chains that target substrate proteins for proteasomal degradation and the K63-linked chains implicated in various signaling pathways (29). K6-linked chains are also generated by BRCA1 autoubiquitination in vivo (28), and they appear to aggregate at sites of DNA damage (30). However, the functional consequences of BRCA1 autoubiquitination and the cellular role of K6-linked polyubiquitin chains are still unclear.

Early studies established that BRCA1 expression fluctuates in a cell cycle-dependent manner. While the steady-state levels of BRCA1 polypeptides are low in resting (G0) cells and G1 cycling cells, these levels increase considerably as cycling cells enter S phase (31, 32). The induction of BRCA1 protein expression that occurs near the G1/S boundary is probably driven by transcriptional activation, since it is preceded by a sharp increase in the levels of BRCA1 mRNA that begins in late G1 (33, 34). Indeed, the promoter region of BRCA1 harbors binding sites for E2F transcription factors that may be responsible for the cell cycle-dependent induction of BRCA1 transcription (35). However, the mechanisms by which BRCA1 levels are down-
regulated as cells progress from S phase to the next G1 phase have not been examined. Here we show that, after peaking in late G1, the steady-state levels of BRCA1 mRNA steadily decline as cycling cells divide and enter the subsequent G1 phase. In addition, we find that BRCA1 polypeptides are subject to ubiquitination and proteasome-mediated degradation at specific stages of cell cycle progression and that dimerization with Ubiquitin is induced in vitro. These data indicate that the cell cycle regulation of BRCA1 expression is determined in part by active degradation through the ubiquitin/proteasome pathway.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The T24, HeLa, and 293 cell lines were obtained from the American Type Tissue Culture Collection and maintained in McCoy's 5A, Dulbecco's modified Eagle's medium, and Iscove's media, respectively, supplemented with 100 μg/ml penicillin/streptomycin, 2 mM L-glutamine, and 10% fetal bovine serum. The following drugs were obtained from Calbiochem and used at the noted concentrations unless otherwise specified: cycloheximide (100 μg/ml), MG132 (20 μM), ALLN (100 μM), ALLM (100 μM), and lactacystin (25 μM). Cells were treated with drugs at ~50% confluence.

**Western Analysis and Antibodies**—Samples were prepared for SDS-PAGE with 10% SDS-containing protein loading dye (0.313 × Tri-HCl, pH 6.8, 10% SDS, 50% glycerol, 25% 2-mercaptoethanol, 0.5% bromophenol blue) and denaturing at 70 °C for 10 min. For Western analysis of BRCA1, 20-μg aliquots of total cell lysate were separated by SDS-PAGE on 3-8% Tris-acetate gels (Invitrogen) run at 135 V for 2 h. For analysis of PTG7, samples were separated on 4-12% Bis-Tris gels (Invitrogen) in MES buffer, where analyses of other proteins were performed using 8-20% Tris-glycine gels. The BARD1-specific polyclonal antiserum (15) and monoclonal antibody (36) have been described. Other antibodies used include BRCA1 (Santa Cruz C-20), cyclin A (Oncogene Ab-3), cyclin B1 (BD Pharmingen), PTG7 (Zymed Laboratories Inc.), α-tubulin (Oncogene Ab-1), PAb1 (Santa Cruz H-152), and ubiquitin (Santa Cruz PDI).

**Cell Cycle Analysis**—T24 cells were arrested in G1 by contact inhibition as described (36). HeLa cells were synchronized in prometaphase by a thymidine/nocodazole block that entailed a 12 h treatment with 2 mM thymidine followed by a 14-18 h treatment with 50 μg/ml nocodazole; the mitotic cells were then harvested by shake-off, and released into nocodazole-free medium after washing twice with phosphate-buffered saline and once with complete Dulbecco's modified Eagle's medium. Both the attached and unattached cells were harvested at each time point and used for FACS, Western, and Northern analyses. For FACS, the cells were stained with propidium iodide as described (36). HeLa cells were treated with MG132 or the specified drug for an additional 5 h. The cells (~2 × 10^6 cells at ~70% confluence) were then lysed by a brief sonication in 700 μl of FLG lysis buffer (50 mM Tris-HCl, pH 7.9, 137 mM NaCl, 1% Triton X-100, 0.2% Sarkosyl, 10% glycerol) supplemented with 1 mM dithiothreitol, protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, BMB protease inhibitor pellet, phosphatase inhibitors (10 mM NaF, 1 mM NaVO₄), and deubiquitination inhibitors (5 mM N-ethylmaleimide, 10 mM ubiquitin aldehyde). Immunoprecipitations were performed by rotating 630 μl of cell lysate with 50 μl of anti-FLAG M2 beads (Sigma, 20% slurry) at 4 °C for 10-12 h. The beads were washed four times with FLG lysis buffer and vortexed for 12 h at 4 °C with 500 μg/ml FLAG peptide in 40 μl of FLG lysis buffer. Samples were separated on 3-8% Tris-acetate gels (Invitrogen), and Western analyses were performed with antibodies that recognize BRCA1 (Oncogene Ab-1), BARD1 (polyclonal 669D), and ubiquitin (Santa Cruz PDI).

**Purification of Endogenous Ubiquitinated Proteins**—To generate a cell line that stably expresses His₆-tagged ubiquitin, HeLa cells were transfected using LipofectAMINE (Invitrogen) with a mammalian expression plasmid (His-Ub, pCIN4) that encodes a polyprotein comprised of 8 tandem copies of His₆-Ub; this plasmid was generated by excising the polyprotein cDNA sequence from pMT107 (37) and inserting it into the pCIN4 vector (pIRES-neo; Clontech). HeLa cell transformants were selected with 0.5 μg/ml G418, and maintained in 0.25 μg/ml G418. The thymidine/nocodazole block and release were performed as described above, with only unattached cells collected at the mitotic time points and only attached cells at the interphase time points. Double thymidine blocks were performed by seeding 2 × 10⁶ cells onto each 100-mm plate, culturing the cells in 2 ml thymidine for 14 h, releasing the cells in thymidine-free medium for 10 h, treating for 2 h with nocodazole, and releasing into nocodazole-free medium containing 50 μg/ml nocodazole. GM132 and cell collection were performed at the time points described (six 100-mm plates at each time point for nocodazole release, four 100-mm plates for double thymidine release). Approximately 1 × 10⁶ cells from each time point were used for FACS analysis, and the remaining cells were lysed in 10 ml of lysis buffer (6 μg guanidine-HCl, 100 mM NaH₂PO₄, 10 mM Tris-HCl, 10 mM imidazole, 10 mM β-mercaptoethanol, pH 8.0) and sonicated for 2 min on ice. To analyze the lysates directly by Western blotting, trichloroacetic acid precipitation was performed by dialuting 10 μl of lysis into 500 μl of water containing 80 μg/ml sodium deoxycholate as a carrier. After adding 71 μl of 100% trichloroacetic acid, the sample was incubated on ice for 1 h and then microcentrifuged for 15 min. The pellet was washed twice with cold acetone, dried, resuspended in cold acetone, and dried again. Western analyses were performed using antibodies that recognize ubiquitination-conjugated proteins, the remainder of the cell lysate was incubated with 50 μl of Ni-NTA agarose beads (Qiagen) for 2 h. The beads were then washed eight times for 5 min each with 4 ml of buffer containing 100 mM NaH₂PO₄, 10 mM Tris-HCl, 10 mM imidazole, 10 mM β-mercaptoethanol supplemented with the following: 1) 6 μg guanidine-HCl, pH 8.0, 2) 6 μM urea, pH 8.0, 3) 8 μM urea, 0.2% Triton X-100, pH 6.3, 4) 8 μM urea, pH 6.3, 5) 8 μM urea, 0.1% Triton X-100, pH 6.3, 6) 8 μM urea, pH 6.3, 7) 8 μM urea, 0.5 μM NaCl, pH 6.3, or 8) 8 μM urea, pH 6.3. The ubiquitin conjugates were then eluted with 50 μl NTA elution buffer (0.15 M Tris-HCl pH 6.7, 200 mM imidazole, 5% SDS, 0.72 mM β-mercaptoethanol, 30% glycerol). After adding 50 protein loading dye and denaturing at 70 °C for 10 min, the eluates were fractionated by SDS-PAGE and analyzed by Western blotting.

**RNAi Transfections**—The siRNAs specific for human BACH1 (AGCUUUACCGUGAACGACGUTdTdT) (38) and human BARD1 (AAGCUAUAACGUGCCAGUAAdTdT) were synthesized by Dharmacon RNA Technologies (Lafayette, CO). In addition, a SMARTpool of four siRNAs specific for BARD1 was also obtained from Dharmacon. HeLa cells were grown in 6-well plates in complete Dulbecco's modified Eagle's medium without antibiotics. At 30% confluence, the cells were transfected with 80 pmol of LipofectAMINE 2000 (Invitrogen) according to the manufacturer's protocol. After 6 h, the transfection mixtures were replaced with complete medium. After culturing the cells for an additional 18 h, the transfection procedure was repeated, and the cells were harvested for analysis 48 h after the initial transfection.

The abbreviations used are: MES, 3-morpholinepropanesulfonic acid; FACS, fluorescence-activated cell sorter; NTA, nitrilotriacetic acid.
RESULTS

Cell Cycle Regulation of BRCA1 and BARD1—To confirm the cell cycle regulation of BRCA1 and BARD1 expression, T24 bladder carcinoma cells were arrested in G0 by contact inhibition, released into fresh medium, and collected at various time points. In accord with previous studies (31, 32), BRCA1 polypeptides are undetectable in arrested T24 cells but begin to appear by 16-h post-induction as cells approach the G1/S transition (Fig. 1A). The levels of BRCA1 increase further during S and G2/M, before decreasing as the cells enter the next G1 phase by 36 h after induction. BRCA1 exists predominantly in a hypophosphorylated state in G1, but becomes hyperphosphorylated in S and G2/M, and reverts to a hypophosphorylated form again in the next G1 phase (31, 32, 39, 40). As reported previously (33, 34), expression of BRCA1 mRNA is maximal in late G1 and decreases as the cells traverse the cell cycle and enter the next G1 phase (Fig. 1B).

Using a BARD1-specific polyclonal antibody, we find that BARD1 protein expression is also regulated with respect to the cell cycle (Fig. 1A). Protein levels follow a similar pattern as for BRCA1, appearing in late G1, increasing during S and G2/M, and decreasing as the cells enter the next G1 phase. The cell cycle-dependent nature of BARD1 protein expression differs from that described previously using a monoclonal antibody raised against BARD1 (36); this discrepancy arose because the monoclonal antibody cross-reacts with a nonspecific band that co-migrates with BARD1 (see Supplementary Data and Discussion). The mRNA expression of BARD1 follows the same pattern as that of BRCA1, peaking in late G1 and decreasing during the remainder of the cell cycle (Fig. 1B). Thus, BRCA1 and BARD1 expression are co-induced at both the mRNA and protein level as cells progress from G1 to G1 phase.

To determine the timing and kinetics of the decrease in BRCA1 and BARD1 protein levels from the G2/M to G1 phase, HeLa cells were arrested in prometaphase by a thymidine/nocodazole block, released into nocodazole-free medium, and collected at hourly time points. Upon release from mitotic arrest, we observed a steady decrease in the levels of BARD1 and especially BRCA1 (Fig. 2A). Although the steady-state levels of their mRNAs also decline after mitosis (Fig. 2B), this result suggests that BRCA1 and BARD1 polypeptides are actively degraded in vivo. Therefore, the kinetics of BRCA1 and BARD1 expression were compared with those of other proteins known to be proteolyzed during mitotic progression, including factors that begin to degrade in prometaphase (cyclin A), at the metaphase/anaphase transition (cyclin B1 and the securin PTTG), and at the M/G1 transition (Plk1) (41). As seen in Fig. 2A, the decline in BRCA1 and BARD1 levels starts later (2–3 h after nocodazole release) than that of cyclin B1 or PTTG (1–2 h after release), and continues into the next G1 phase. This pattern more closely resembles that of Plk1, a protein kinase that undergoes proteasome-mediated degradation beginning at the M/G1 transition (42, 43).

BRCA1 Degradation Is Mediated by the Proteasome—To test whether endogenous BRCA1 and BARD1 polypeptides are actively degraded, their steady-state levels in asynchronous 293 cells were monitored after treatment with cycloheximide, an inhibitor of protein synthesis. As shown in Fig. 3A, BRCA1 protein levels declined within a few hours and displayed a much shorter half-life than a-tubulin. This implies that proliferating 293 cells possess an active mechanism for BRCA1 deg-
polypeptides in 293 cells treated with 100 μg/ml cycloheximide for various lengths of time. B, 293 cells were co-incubated with 100 μg/ml cycloheximide and various concentrations of the protease inhibitors MG132, ALLN, ALLM, and lactacystin. Cells were harvested after 8 h of treatment, and lysates were analyzed for levels of endogenous BRCA1, BARD1, and α-tubulin polypeptides by Western analysis.

Exogenous BRCA1 Polypeptides Are Subjected to Proteasome-sensitive Polyubiquitination in Vivo—Since BRCA1 is autoubiquitinated during BRCA1/BARD1-dependent in vitro ubiquitination reactions (22), the in vivo ubiquitination of BRCA1 detected in our assay may be due to the catalytic activity of BRCA1 itself or to that of a distinct cellular E3 ligase(s). Nishikawa et al. (28) recently demonstrated that a truncated form of BRCA1 (amino acid residues 1–772) can catalyze autoubiquitination through lysine 6 of ubiquitin in vitro and showed that the levels of autoubiquitinated BRCA1 are not enhanced in the presence of a proteasome inhibitor. To address whether the ubiquitination detected in our assay is also due to autoubiquitination, we expressed a similar BRCA1 segment (ΔBRCA1, amino acids 1–771) in the presence of BARD1 and either His6-Ub (as in our previous experiments) or HA-Ub (as in those of Nishikawa et al.). We then compared the in vivo ubiquitination of FLAG-tagged ΔBRCA1 segments that do or
do not harbor the I26A mutation, an amino acid substitution that impairs the catalytic activity of BRCA1 by disrupting its interaction with cognate E2 enzymes (46). Lysates of transfected 293 cells were immunoprecipitated with FLAG-specific antibodies, and the presence of FLAG-tagged ΔBRCA1 and its ubiquitin conjugates was measured by immunoblotting with BRCA1-specific (lanes 1–4) and Ub-specific (lanes 5–8) antibodies, respectively. As shown in Fig. 6, the steady-state levels of the wild-type (lanes 1 and 2) and mutant (lanes 3 and 4) ΔBRCA1 segments increase modestly in the presence of the proteasome inhibitor MG132. A ladder of ubiquitinated ΔBRCA1 forms is also apparent in these lanes, but it is more prominent with wild-type ΔBRCA1 (lanes 1 and 2) than with the corresponding I26A mutant (lanes 3 and 4). Likewise, when detected by immunoblotting with Ub-specific antibodies, the levels of ubiquitinated conjugates are also clearly more abundant with wild type (lane 5) than mutant (lane 7) ΔBRCA1. In this respect, our data are consistent with the previous results of
Nishikawa et al. (28), and support their contention that BRCA1 undergoes autoubiquitination in vivo. However, we also observe that low steady-state levels of ubiquitinated conjugates are formed from the catalytically impaired ∆BRCA1 segment (lane 7). Moreover, because the levels of these conjugates are dramatically enhanced in the presence of a proteasome inhibitor (compare lanes 7 and 8), they are likely to serve as substrates for proteasomal degradation. Therefore, in addition to autoubiquitination, ∆BRCA1 is also subjected to transubiquitination in vivo, presumably by cellular E3 ligases that promote BRCA1 turnover.

To address whether full-length BRCA1 polypeptides are also subject to ubiquitination, we transfected 293 cells with expression vectors that encode full-length BRCA1 bearing either the wild-type sequence, the catalytically impaired I26A mutation, or the C61G missense mutation—a cancer-predisposing lesion that also ablates the enzymatic activity of BRCA1. As shown in Fig. 7, in vivo ubiquitination of the mutants is only modestly reduced relative to that of wild-type BRCA1 (lanes 13, 15, and 17). Thus, under these conditions the polyubiquitination of full-length BRCA1 polypeptides is largely independent of its own enzymatic activity.

In addition to inactivating the catalytic activity of BRCA1, the C61G mutation also impairs its in vivo interaction with BARD1 (15). In contrast, the I26A mutation does not affect formation of the BRCA1/BARD1 heterodimer (46). As shown in Fig. 7, co-expression of BARD1 with either wild-type BRCA1, C61G-BRCA1, or I26A-BRCA1 increases the levels (lanes 8, 10, and 12) and decreases the ubiquitination (lanes 14, 16, and 18) of each of these polypeptides. However, the effect of BARD1 expression on the C61G mutant (lanes 10 and 16) is less pronounced than for either wild-type BRCA1 (lanes 8 and 14) or the I26A mutant (lanes 12 and 18), likely because of its weaker interaction with BARD1.

Endogenous BRCA1 Polypeptides Are Also Subject to Proteasome-sensitive Polyubiquitination—We have demonstrated that exogenously expressed BRCA1 polypeptides can be ubiquitinated in vivo (Figs. 4–7). To determine whether endogenous BRCA1 can also be ubiquitinated, we created a stable line of HeLa cells that expresses His$_6$-tagged ubiquitin (Fig. 8A); as a result, ubiquitin-conjugated cellular proteins can be purified from lysates of these cells by affinity chromatography on nickel agarose (NTA) beads. The His$_6$-Ub expressing cells and the parental HeLa cells were cultured in the presence or absence of MG132 and lysed under denaturing conditions. Ubiquitinated BRCA1 polypeptides were then recovered on NTA beads, and detected by immunoblotting with BRCA1-specific antibodies. As seen in Fig. 8B, trace amounts of BRCA1 bind non-specifically to the NTA beads, even from parental HeLa cells that do not express His$_6$-Ub (lanes 1 and 3). In addition, however, slower migrating forms of BRCA1 are readily detected in the His$_6$-Ub expressing cells (lane 5), and the appearance of these forms is enhanced by the addition of a proteasome inhibitor (lane 7). Thus, endogenous BRCA1 can be ubiquitinated in vivo, and at least some of this ubiquitination is associated with proteasomal degradation.

In Vivo Ubiquitination of Endogenous BRCA1 Is Regulated With Respect to the Cell Cycle—BRCA1 protein expression is cell cycle regulated, with a steady decrease in levels as cells exit mitosis (Fig. 2A). Whereas the concomitant reduction in mRNA levels suggests a transcriptional component to this decrease (Fig. 2B), we sought to determine whether ubiquitin-mediated proteasomal degradation also plays a role. Thus, HeLa cells that stably express His$_6$-Ub were arrested in prometaphase by thymidine/nocodazole block and released into nocodazole-free medium for various lengths of time (0–20 h), after which the cells were incubated with MG132 for an additional 6 h. The cells were then lysed and the ubiquitinated proteins were recovered by NTA chromatography as described above. As a control, we also monitored the ubiquitination of PTTG, a mammalian securin whose kinetics of degradation during mitosis have been well characterized (47). Proteasomal degradation of PTTG is required for the activation of separase, a protease that cleaves the cohesins that link sister chromatids. As a result, metaphase cells treated with a proteasome inhibitor do not progress into anaphase because the sister chromatids cannot separate. Therefore, cells released directly from nocodazole block into medium containing MG132 will arrest in metaphase and accumulate ubiquitinated PTTG that cannot be degraded. As shown in Fig. 8C, we do not detect ubiquitinated PTTG in cells arrested in prometaphase by nocodazole (lane 1). As expected, however, ubiquitinated forms of PTTG accumulate in cells arrested in metaphase (lane 3), but disappear if these cells are allowed to progress into the next G1 phase prior to MG132 treatment (lanes 5 and 7).

BRCA1 ubiquitination follows a different pattern from that of PTTG (Fig. 8C). Although some unmodified BRCA1 from each time point binds non-specifically to the NTA beads, little, if any, of the slowly migrating ubiquitinated forms of BRCA1 are seen in cells arrested in prometaphase (Fig. 8C, lane 1) or
Fig. 8. Cell cycle-regulated ubiquitination of endogenous BRCA1. A, lysates of parental HeLa cells (lane 1) and HeLa cells that stably express a His$_{6}$-tagged ubiquitin (His-Ub cells, lane 2) were analyzed by immunoblotting with a ubiquitin-specific antibody. B, asynchronous populations of HeLa cells (lanes 1–4) and His-Ub cells (lanes 5–8) were treated with Me$_{6}$SO (lanes 1–2, 5–6) or with 20 µM MG132 (lanes 3–4, 7–8) for 6 h. Ubiquitin-conjugated polypeptides were then purified from the cell lysates by binding to Ni-NTA agarose beads and eluting in a buffer containing imidazole. Western analyses of the chromatography eluates (E), and the untreated cell lysates (L) were performed using antibodies against BRCA1 and ubiquitin. C, His-Ub cells were arrested in prometaphase by thymidine/nocodazole block and released into nocodazole-free medium for various lengths of time (0–20 h), after which the cells were incubated with MG132 for an additional 6 h. The harvested cells were then analyzed by FACS, and cell lysates were subjected to Ni-NTA chromatography as in B. Western analyses of the chromatography eluates (E), and untreated cell lysates (L) were conducted with antibodies specific for BRCA1 and PTTG. Asterisks denote two bands that non-specifically bound the Ni-NTA beads and are recognized by the PTTG antibody.

High Turnover of Endogenous BRCA1 Polypeptides during S phase—To determine whether the S phase-specific ubiquitination of BRCA1 is also related to proteasomal degradation, His$_{6}$-Ub expressing HeLa cells were arrested at the G$_{1}$/S boundary by a double thymidine block and then released into fresh medium containing nocodazole (to prevent cells from progressing into the subsequent G$_{2}$ phase). At 4-hour intervals after release, parallel cultures were either harvested immediately or treated with MG132 for an additional 6 h. As the cells traverse S phase, ubiquitinated forms of BRCA1 are much more prominent in cells treated with a proteasome inhibitor (Fig. 9, lanes 10 and 12) than without (lanes 9 and 11), implying that the S phase ubiquitination of BRCA1 is indeed associated with proteasomal degradation. The levels of ubiquitinated BRCA1 steadily decrease as the cells progress from G$_{2}$/S (lanes 10 and 12) to G$_{2}$/M (lanes 14 and 16) while protein levels progressively increase (lanes 1, 3, 5, and 7), suggesting that BRCA1 is stabilized as cells enter mitosis. Interestingly, the ubiquitination and proteasomal degradation of BRCA1 seems most active around early S phase, a stage of the cell cycle when BRCA1 mRNA levels are elevated and BRCA1 protein begins to accumulate (Fig. 1). Thus, the results of Figs. 8 and 9 indicate that turnover of the BRCA1 protein is greatest, and its expression is most tightly regulated, during S phase, but that the formation of proteasome-sensitive ubiquitinated conjugates of BRCA1 is reduced or ceases in nocodazole-arrested cells.

Interestingly, after proliferating HeLa cells are subject to 6 h of treatment with MG132, most BRCA1 polypeptides exist in a hypophosphorylated state (Fig. 8B, lanes 4 and 8). We also observe this phenomenon after MG132 treatment of cells synchronized (Fig. 9, lane 2) or released (lanes 4 and 6) from a G$_{2}$/S block, and some hypophosphorylated BRCA1 appears after MG132 treatment even in mitotic cells (lane 8). This may occur because 1) the proteasome is responsible for degradation of either BRCA1 phosphatases or BRCA1 kinase inhibitors, 2) MG132 has effects on cellular BRCA1 in HeLa cells that are independent of its inhibition of the proteasome, or 3) hypophosphorylated BRCA1 is more susceptible to proteasomal degradation than hyperphosphorylated BRCA1. The latter possibility is consistent with the data of Fig. 3A in which hypophosphorylated BRCA1 decays faster than hyperphosphorylated BRCA1 in 293 cells treated with cycloheximide.

In cells arrested at prometaphase by nocodazole, BRCA1 exists as a single band (Fig. 10, lane 2) that migrates with a
quantities of lysate were fractionated from asynchronous cells (20/H9262 specific antibody. To obtain bands of comparable intensity, different and lanes 2 released from a thymidine/nocodazole block for 0, 2, and 5 h (lanes 2, 4, and 6, respectively) were examined by immunoblotting with a BRCA1-specific antibody. To obtain bands of comparable intensity, different quantities of lysate were fractionated from asynchronous cells (20 μg) and from cells released for 0 h (2.0 μg), 2 h (2.5 μg), and 5 h (5.0 μg).

mobility intermediate to those of the hyper- and hypophosphorylated BRCA1 species detectable at other cell cycle stages (lanes 1 and 3). The BRCA1 polypeptides of mitotic cells presumably bear a distinctive pattern of post-translational modifications distinct from those of the hypo- and hyperphosphorylated BRCA1 (48). If the hypophosphorylated forms of BRCA1 are indeed more susceptible to proteasomal degradation, then the absence of these forms in prometaphase may explain why ubiquitination of BRCA1 is reduced at this stage of the cell cycle (Fig. 9, lanes 15 and 16).

DISCUSSION

Early studies established that BRCA1 expression is low or undetectable in resting (G0) cells, but that the steady-state levels of its mRNA and protein products increase markedly at the G1/S transition after resting cells are induced to proliferate (31–34). These findings revealed the cell cycle-dependent nature of BRCA1 expression and indicated that transcriptional mechanisms are important for the increase in BRCA1 levels that occurs near the G1/S boundary. Here we show that BRCA1 levels are also regulated with respect to the cell cycle by ubiquitin-dependent proteolysis. Blagosklonny et al. (49) had previously examined the mechanism of BRCA1 turnover in cell lines expressing basal levels of BRCA1 protein that are low or undetectable in cycling cells, and found that BRCA1 degradation in these cells does not depend on the proteasome but is instead mediated primarily by a caspase-like protease(s). In contrast, it was observed that the steady-state levels of BRCA1 are increased upon treatment of 293 cells with the proteasomal inhibitor MG132 (45). While other proteases may also contribute to degradation of cellular BRCA1 polypeptides, our demonstration that ubiquitinated forms of BRCA1 exist in HeLa and 293 cells, and that their levels are elevated by proteasome inhibition, indicates that the ubiquitin/proteasome pathway also serves to regulate BRCA1 expression in vivo.

When unsynchronized proliferating cell populations are examined by immunostaining, the levels of BRCA1 protein appear to be significantly higher in S and G2 phase cells than in G1 cells (40). This implies that BRCA1 expression is down-regulated as cells progress from G2 to the subsequent G1 stage. However, the timing and nature of this down-regulation had not been addressed. By monitoring synchronized cells released from mitotic arrest, we now show that the steady-state levels of BRCA1 polypeptides start to decline around the M/G1 transition (Fig. 2A). Parallel analysis of other proteins known to be proteolyzed at specific times during mitosis indicates that BRCA1 down-regulation begins subsequent to prometaphase (marked by the onset of cyclin A degradation) and the metaphase/anaphase transition (cyclin B1 and securin/PTTG degradation) and coincident with the proteolysis of Plk1, a protein kinase that undergoes ubiquitin-dependent degradation beginning at the M/G1 transition (42, 43). Our data also suggest that at least two processes can potentially contribute to cell cycle-associated BRCA1 down-regulation. First, the levels of BRCA1 mRNA continue to decline after peaking in S phase, perhaps reflecting decreased initiation of BRCA1 transcription and/or increased turnover of BRCA1 mRNA (Fig. 1B). Second, ubiquitinated conjugates of endogenous BRCA1 polypeptides begin to appear as cells proceed from mitosis to the subsequent G1 phase (Fig. 8). Since these conjugates accumulate markedly in cells treated with specific proteasome inhibitors, they are likely to reflect increased proteolysis of BRCA1 at the M/G1 transition and probably contribute to the down-regulation of BRCA1 expression that occurs in early G1.

Because the steady-state levels of BRCA1 protein increase at the G1/S transition (31, 32), we had expected that the levels of proteasome-sensitive ubiquitinated BRCA1 conjugates would decrease as cycling cells progressed from G1 to the subsequent S phase. Surprisingly, however, these levels remained high throughout S phase, and did not decrease until G2 or M (Fig. 8). This indicates that high rates of BRCA1 ubiquitination and degradation are occurring during S phase, a stage of cell cycle progression when the steady-state levels of BRCA1 are high. BRCA1 has been implicated in a number of important S phase functions, such as homology-directed DNA break repair (11–13) and the DNA damage-induced S phase checkpoint (5). Thus, it is not obvious why BRCA1 synthesis and degradation should be elevated simultaneously in S phase. The high rate of BRCA1 turnover in S phase may allow for more stringent control of BRCA1 activity by promoting rapid changes in BRCA1 levels, localization, and/or post-translational modification. In any case, these data reveal that BRCA1 expression is controlled in part by the ubiquitin/proteasome pathway and that turnover of BRCA1 polypeptides is elevated during S phase.

In contrast to G1 and S phase cells, ubiquitin conjugates of endogenous BRCA1 are barely detectable in cells arrested at prometaphase by nocodazole (e.g. see lane 1, Fig. 8C). This implies that BRCA1 ubiquitination is suspended during mitosis, at least while the spindle checkpoint is enforced. It appears that BRCA1 ubiquitination is also reduced during subsequent stages of mitosis since the level of ubiquitinated BRCA1 conjugates remains low after cells are released from nocodazole arrest in the presence of proteasome inhibitor (lane 3, Fig. 8C), a treatment that blocks cells in a subsequent stage of mitosis, but terminates the spindle checkpoint as manifested by the ubiquitination of the securin PTTG. Thus, although ubiquitination and degradation of BRCA1 occurs during G1 and S, it is specifically suspended during mitosis. This mitotic stabilization of BRCA1 is intriguing given recent evidence that BRCA1 performs important functions during mitosis. In particular, Brca1-deficient mouse embryonic cells display mitotic lesions, including spindle abnormalities and centrosome amplification (6, 50), and BRCA1 polypeptides are reported to bind γ-tubulin and localize to the centromeres and spindle microtubules of mitotic cells (51–54). Moreover, when Brca1- or Bard1-null blastocysts are cultured in vitro, cells of the inner cell mass fail to proliferate, while trophoblast giant cells, which endoreplicate their DNA without undergoing mitosis, appear to be unaffected (16, 55). These observations indicate that BRCA1 has critical functions in mitosis, and suggest that disruption of these functions may be responsible for some aspects of the genomic instability that characterize Brca1-deficient cells (6, 50).

In vitro ubiquitination reactions driven by the BRCA1/BARD1 heterodimer generate conjugates of BRCA1 that harbor primarily K6-linked polyubiquitin chains (27, 28). Nishikawa et al. (28) have shown that K6-linked polyubiquitin conjugates of BRCA1 are also produced in vivo by autoubiquitination in
cells transfected with exogenous BRCA1 polypeptides. In addition, they found that the levels of these conjugates are not affected by proteasome inhibitors (28). Our present data indicate that exogenous BRCA1 can also be polyubiquitinated in vivo in a manner independent of its own enzymatic activity (Figs. 6 and 7), presumably through the action of other cellular E3 ligases. The resulting ubiquitin conjugates are likely to serve as intermediates for proteasomal degradation of BRCA1 since they can be stabilized by treatment with proteasome inhibitors. Indeed, by using cells that express epitope-tagged ubiquitin, we were able to detect ubiquitinated conjugates of endogenous BRCA1 and show that their levels are also enhanced by proteasome inhibition (Fig. 8B). Thus, the in vivo pool of ubiquitinated BRCA1 species appears to include both proteasome-insensitive and proteasome-sensitive conjugates.

On one hand, the proteasome-insensitive BRCA1 conjugates are generated by autoubiquitination, a process that generates K6-linked chains and may impart critical, but as yet unknown, effects on BRCA1 activity (28). On the other hand, the proteasome-sensitive conjugates represent BRCA1 intermediates destined for degradation and as such are likely to harbor ubiquitin modifications that are recognized by the proteasome, such as K48-linked chains. Interestingly, the balance between proteasome-insensitive and proteasome-sensitive conjugates differed depending on the nature of the BRCA1 polypeptide under analysis. While proteasome-insensitive conjugates were prominent in cells transfected with truncated BRCA1 (28) (Fig. 6), proteasome-sensitive conjugates were more abundant in cells transfected with full-length BRCA1 (Fig. 7) and upon analysis of endogenous BRCA1 (Fig. 8). Although we do not know the basis for this phenomenon, the truncated BRCA1 polypeptides may represent enzymatically-deregulated forms with a heightened capacity for autoubiquitination.

Previous studies had shown that BRCA1 and BARD1 exert a reciprocal stabilizing effect on one another, such that expression of one subunit of the heterodimer serves to increase the steady-state levels of the other subunit (16, 21, 45). In light of this phenomenon, as well as the fact that the enzymatic activity of the heterodimer is dramatically higher than that of either BRCA1 or BARD1 alone (21), it seems unlikely that BRCA1 degradation would be triggered by autoubiquitination. Instead, the proteasome-sensitive ubiquitin conjugates of BRCA1 are probably generated by other cellular E3 ligases. At present, however, we do not know which E3 ligases are responsible for BRCA1 degradation. The anaphase promoting complex (APC) is an intriguing candidate since the kinetics of BRCA1 decay at the M/G1 transition are reminiscent of late APC substrates, such as Plk1 and Cdc20 (41). However, in contrast to these proteins, proteasome-sensitive ubiquitination of BRCA1 continues as cells progress into S phase and does not cease until the following G2 or M phase. Although other cell cycle-regulated E3 ligases, such as the skp1/cullin/F-box (SCF) complex, might promote BRCA1 ubiquitination in S phase, further studies will be required to identify the cellular enzymes responsible for BRCA1 ubiquitination. Indeed, these enzymes may be relevant in sporadic cases of breast cancer. While BRCA1 mutations are not observed in sporadic breast cancer, the tumor cells of these patients often exhibit reduced steady-state levels of BRCA1 polypeptides (56). In some cases this has been attributed to impaired synthesis due to hypermethylation of the BRCA1 promoter. However, an increased rate of BRCA1 degradation, conceivably mediated by a deregulated E3 ligase, might constitute another mechanism by which BRCA1 levels are reduced in sporadic breast cancer.

It is intriguing to note that the formation of proteasome-sensitive ubiquitin conjugates of BRCA1 is reduced by co-expression of BARD1 (Fig. 7). Although we do not know the molecular mechanism by which this is achieved, the ability of BARD1 to protect BRCA1 polypeptides from ubiquitination may account in part for the mutual stabilization that is observed upon co-expression of BRCA1 and BARD1 (16, 21, 45).

The results of Fig. 1, which were obtained by immunoblotting cell lysates with a BARD1-specific polyclonal antiserum, demonstrate that BARD1 protein expression is regulated with respect to the cell cycle. However, our previous data using a monoclonal antibody raised against BARD1 did not reveal cell cycle fluctuations in BARD1 protein levels by immunoblotting, despite the fact that immunofluorescence analyses with the same antibody, as well as with the BARD1-specific polyclonal antiserum, had shown perfect co-localization of BRCA1 and BARD1 polypeptides during S phase but little, if any, BARD1 staining during G1 (36). We now demonstrate that this discrepancy arose because the monoclonal antibody cross-reacts strongly with an unrelated species in Western blots of human cell lysates; this species co-migrates with BARD1 during SDS-PAGE and its levels do not change during cell cycle progression (Supplemental Figs. A and B). However, when the same analysis is conducted with a polyclonal antiserum that specifically recognizes BARD1, the steady-state levels of BARD1 are found to fluctuate with cell cycle progression in parallel with those of BRCA1, at least with respect to the G1/S induction (Fig. 1). Interestingly, however, the half-life of BARD1 appears to be longer than that of BRCA1, indicating a higher turnover of BRCA1 than BARD1 in asynchronously cycling cells (Fig. 3). This implies that BRCA1 is the more tightly controlled subunit of the BRCA1/BARD1 heterodimer and raises questions as to how and when regulation of the two subunits is accomplished differently. While we have been able to demonstrate in vivo ubiquitination of exogenous BARD1 polypeptides (data not shown), we have not successfully detected ubiquitinated conjugates of endogenous BARD1, possibly due to the limitations of current BARD1 antibodies. Thus, it is unclear whether the same active mechanisms that degrade BRCA1 also apply to BARD1 or whether the more gradual decline in BARD1 levels during G1 reflects a passive reaction to changes in BRCA1 levels.

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REFERENCES

1. Wooster, R., and Weber, B. L. (2003) N. Engl. J. Med. 348, 2339–2347
2. Moynahan, M. E. (2002) Oncogene 21, 8994–9007
3. Jasin, M. (2002) Oncogene 21, 8981–8990
4. Powell, S. N., and Kachnic, L. A. (2003) Oncogene 22, 5784–5791
5. Xu, B., Kim, S., and Kastan, M. B. (2001) Mol. Cell. Biol. 21, 3445–3450
6. Xu, X., Weaver, Z., Linke, S. P., Li, C., Gotay, J., Wang, X. W., Harris, C. C., Bied, T., and Deng, C. X. (1999) Mol. Cell. Biol. 19, 2039–2046
7. Kleinman, F. E., and Manley, J. L. (1999) Cancer Cell 2, 389–399
8. Kleiman, F. E., and Manley, J. L. (1999) Science 285, 1576–1579
9. Abbott, D. W., Thompson, M. E., Robinson-Beninc, C., Tomlison, G., Jensen, R. A., and Holt, J. T. (1999) J. Biol. Chem. 274, 18808–18812
10. Le Page, F., Randrianarison, V., Marot, D., Cabannes, J., Perricaudet, M., Feunteun, J., and Sarasin, A. (2000) Cancer Res. 60, 5548–5552
11. Moynahan, M. E., Chiu, J. W., Keller, B. H., and Jasin, M. (1999) Mol. Cell 4, 511–518
12. Moynahan, M. E., Cui, T. Y., and Jasin, M. (2001) Cancer Res. 61, 4842–4850
13. Sweetort, J. N., Gowen, L. C., Latour, A. M., Mohn, A. R., Xiao, A., DiBiase, R., Xian, A., DiBiase, L., and Keller, B. H. (1999) Oncogene 18, 7900–7907
14. Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Linch, D. C., Bennett, S., Ding, W., Ball, R., Rosenthal, J.; Hussey, C., Tran, M., McClure, M., Frye, C., Hattier, T., Phelps, R., Haugen-Straano, A., Katcher, H., Yakumo, K., Gholami, Z., Shaffer, D., Stone, S., Bayer, S., Wray, C., Bogden, R., Daynannah, P., Ward, J., Tonin, P., Narod, S., Bratow, P. K., Norris, P. 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,
Ubiquitination and Proteasomal Degradation of BRCA1

M. H. (1994) Science 266, 66–71
15. Wu, L. C., Wang, Z. W., Tsan, J. T., Spillman, M. A., Phung, A., Xu, X. L., Yang, M.-C. W., Hwang, L.-Y., Bowcock, A. M., and Baer, R. (1996) Nature Genet. 14, 430–440
16. McCarthy, E. E., Celebi, J. T., Baer, R., and Ludwig, T. (2003) Mol. Cell. Biol. 23, 5056–5063
17. Westermark, U. K., Reyngold, M., Olshen, A. B., Baer, R., Jasim, M., and Meynahan, M. E. (2003) Mol. Cell. Biol. 23, 7526–7536
18. Brzovic, P. S., Rajagopal, P., Hoyt, D. W., King, M.-C., and Klevit, R. E. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 43918–43923
19. Lorick, K. L., Jensen, J. P., Fang, S., Ong, A. M., Hatakeyama, S., and Weissman, A. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11364–11369
20. Brzovic, P. S., Rajagopal, P., Hoyt, D. W., King, M.-C., and Klevit, R. E. (2001) Nature Struct. Biol. 8, 833–837
21. Lorick, K. L., Jensen, J. P., Fang, S., Ong, A. M., Hatakeyama, S., and Weissman, A. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11364–11369
22. Ruffner, H., Joazeiro, C. A., Hemmati, D., Hunter, T., and Verma, I. M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 5134–5139
23. Kentsis, A., Gordon, R. E., and Borden, K. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 15404–15409
24. Chen, A., Kleiman, F. E., Manley, J. L., Ouchi, T., and Pan, Z. Q. (2002) J. Biol. Chem. 277, 14537–14540
25. Mallery, D. L., Vandenberg, C. J., and Hiom, K. (2002) EMBO J. 21, 6755–6762
26. Ohta, T., and Fukuda, M. (2004) Oncogene 23, 2079–2088
27. Wu-Baer, F., Lagrazon, K., Yuan, W., and Baer, R. (2003) J. Biol. Chem. 278, 34743–34746
28. Nishikawa, H., Oka, S., Sato, K., Arima, K., Okamoto, J., Klevit, R. E., Fukuda, M., and Ohta, T. (2004) J. Biol. Chem. 279, 3916–3924
29. Pickart, C. M. (2000) Trends Biochem. Sci. 25, 544–548
30. Morris, J. R., and Solomon, E. (2004) Hum. Mol. Genet. 13, 807–817
31. Chen, Y., Farmer, A. A., Chen, C.-F., Jones, D. C., Chen, P.-L., and Lee, W.-H. (1996) Cancer Res. 56, 3168–3172
32. Ruffner, H., and Verma, I. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7138–7143
33. Gudas, J. L., Li, T., Nguyen, H., Jensen, D., Rauscher, F. J., III, and Cowen, K. H. (1996) Cell Growth Diff. 7, 717–723
34. Vaughn, J. P., Davis, P. L., Jarboe, M. D., Huger, G., Evans, A. C., Wiseman, R. W., Futreal, P. A., and Marks, J. R. (1996) Cell Growth Diff. 7, 711–715
35. Wang, A., Schneider-Broussard, R., Kumar, A. P., MacLeod, M. C., and Johnson, D. G. (2000) J. Biol. Chem. 275, 4532–4536
36. Jin, Y., Xu, X. L., Yang, M.-C. W., Wei, F., Ayi, T.-C., Bowcock, A. M., and Baer, R. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12075–12080
37. Treiser, M., Stasiewski, L. M., and Bohmann, D. (1994) Cell 78, 782–786
38. Yu, X., Chini, C. C., He, M., Mer, G., and Chen, J. (2003) Science 302, 639–642
39. Thomas, J. E., Smith, M., Tenenbaum, J. L., Rubinfeld, B., and Polakis, P. (1997) Cell Growth Diff. 8, 801–809
40. Scully, R., Chen, J., Ochs, R. L., Keegan, K., Hoeckstra, M., Feunteun, J., and Livingston, D. M. (1997) Cell 89, 425–435
41. Peters, J. M. (2002) Mol. Cell 9, 913–941
42. Fang, G., Yu, H., and Kirschner, M. W. (1998) Mol. Cell 2, 163–171
43. Pellet, D. K., Maloid, S. C., and Li, C. C. (1998) Biochem. Biophys. Res. Commun. 252, 340–344
44. Lee, D. H., and Goldberg, A. L. (1998) Trends Cell Biol. 8, 397–403
45. Ferris, D. K., Maloid, S. C., and Li, C. C. (1998) Biochem. Biophys. Res. Commun. 252, 340–344
46. Lee, D. H., and Goldberg, A. L. (1998) Trends Cell Biol. 8, 397–403
47. Joukov, V., Chen, J., Fox, E. A., Green, J. B., and Livingston, D. M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 12078–12083
48. Brzovic, P. S., Keeffe, J. R., Nishikawa, H., Miyamoto, K., Fox, D., 3rd, Fukuda, M., Ohta, T., and Klevit, R. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 15404–15409
49. Ouchi, M., Fujiuchi, N., Katayama, H., Minamimori, Y. A., Ongusaha, P. P., Deng, C., Sen, S., Lee, S. W., and Ouchi, T. (2004) J. Biol. Chem. 279, 19643–19648
50. Blagosklonny, M. V., An, W. G., Melillo, G., Nguyen, P., Trepel, J. B., and Neckers, L. M. (1999) Oncogene 18, 6469–6486
51. Shen, S. X., Weaver, Z., Xu, X., Li, C., Weinstein, M., Chen, L., Guan, X. Y., Reid, T., and Deng, C. X. (1998) Oncogene 17, 3115–3124
52. Hsu, L. C., and White, R. L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12083–12088
53. Hsu, L. C., Doan, T. P., and White, R. L. (2001) Cancer Res. 61, 7713–7718
54. Lottis, L. V., Ottini, L., D’Amico, C., Gradini, R., Cama, A., Belleudi, F., Frati, L., Torrisi, M. R., and Mariani-Costantini, R. (2002) Genes Chromo. Cancer 35, 193–203
55. Okada, S., and Ouchi, T. (2003) J. Biol. Chem. 278, 2015–2020
56. Ludwig, T., Chapman, D. L., Papaioannou, V. E., and Eleftheriadis, A. (1997) Genes Dev. 11, 1226–1241
57. Mueller, C. R., and Roskelley, C. D. (2003) Breast Cancer Res. 5, 45–52
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