Identification of TopBP1 as a c-Abl-interacting Protein and a Repressor for c-Abl Expression

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Expression of BCR-ABL is the leading cause of chronic myelogenous leukemia. In chronic myelogenous leukemia cells, c-Abl expression is silenced by promoter methylation. In addition, the level of c-Abl needs to be tightly and constantly regulated due to its cytotoxicity and its rapid degradation after activation. Yet the regulation of c-Abl expression remains unclear. In an effort to gain better understanding of c-Abl function, we performed a glutathione S-transferase-Abl pull-down screen and identified TopBP1, a topoisomerase IIβ-binding protein that contains Brcal C-terminal motifs and has been implicated in DNA damage response. Their physical interaction was verified by in vitro and in vivo assays with TopBP1 found as a substrate of Abl proteins. TopBP1 could repress the expression of c-Abl at both mRNA and protein levels. Reporter assays indicate that TopBP1 could directly repress the promoter activity of c-Abl. Furthermore, TopBP1 repressed expression of c-Abl through a novel mechanism that involved histone deacetylation and DNA methylation. This transcriptional repression was inhibited by c-Abl in a kinase-dependent manner. The dual antagonistic interaction between c-Abl and TopBP1 may also provide a mechanism for fine-tuning of c-Abl levels.

The c-Abl proto-oncogene, frequently altered in CML, encodes a non-receptor tyrosine kinase. Reciprocal translocation of chromosomes 9 and 21 in human results in expression of a fusion protein BCR-ABL, which is highly active as a tyrosine kinase and underlies the development of most CML cases (1). Recently, Iamatinib/STI571/Gleevec, a specific inhibitor for Abl, has been found to be clinically effective in CML treatment (2). Recently, Imatinib/STI571/Gleevec, a specific inhibitor for Abl, has been found to be clinically effective in CML treatment (2). Although great progress has been made in understanding the roles of BCR-ABL in the development of CML, the physiological function of c-Abl is still elusive. Accumulating evidence suggests that c-Abl is involved in genotoxic stress response, actin dynamics, and mitogenesis (3–6).

c-Abl has been implicated in DNA damage response (7–9). It is activated by ionizing radiation and radiomimetic reagents in an Atm-dependent manner. In fibroblasts the activated c-Abl may promote apoptosis by stabilizing p73 and/or p53 (10, 11). In addition, c-Abl has been shown to interact with several proteins involved in DNA damage response including DNA-PKcs, WRN, Atm, and Brca1 (12–16). In most cases the biological significance of these interactions remains unclear. In addition, BCR-ABL has been shown to play an important role in DNA damage response (17). Although the molecular mechanisms underlying the participation of c-Abl in DNA damage response remain an enigma, an increase in c-Abl levels via ectopic expression evidently leads to cell cycle arrest and apoptosis (18, 19). The cells have evolved mechanisms to regulate the levels of c-Abl, such as protein degradation by the ubiquitin-proteasome pathway and hypermethylation of c-Abl promoters (20–22). In CML cells, the expression of c-Abl is silenced by DNA methylation at the promoter regions of c-Abl, although the biological significance and the molecular mechanism of the repression are still unclear.

A superfamily of about 40 BRCT-containing proteins has emerged as an important player in genotoxic stress response (23). BRCT domains contain 80–100 amino acid residues and are believed to mediate protein-protein interaction or to be involved in binding phosphorylated peptides (24, 25). Members of this family include Brcal, Brcal, 53BP1, Mdc1, TopBP1, and so on. TopBP1, a yeast cut4/rad5 homologue, has been implicated in cell cycle control, apoptosis, and gene regulation (26, 27). Some of the BRCT-containing proteins, such as Brcal and TopBP1, assemble at the nuclear foci upon DNA damage (28, 29). The c-Abl/TopBP1 interaction appears to play vital roles in differentiation and oxidative stress response in osteoblasts and that c-Abl knock-out mice show osteoporosis (29, 30). TopBP1 was identified as one of the interacting proteins of c-Abl. We demonstrated a constitutive interaction between c-Abl and TopBP1, TopBP1 was an in vivo substrate for c-Abl, especially upon DNA damage. Furthermore, we found that TopBP1 repressed c-Abl expression at the level of transcription, probably by recruiting histone deacetylase 1 (HDAC1). The repressive action of TopBP1 on c-Abl transcription was inhibited by c-Abl but not by kinase dead c-Abl, suggesting that this regulation was phosphorylation-dependent. These findings also imply that a feedback regulation loop exists to modulate c-Abl expression.

MATERIALS AND METHODS

Cell Culture and Transfection—Mouse embryonic fibroblasts were prepared as previously described (30). MEFs, MC3T3-E1, COS7, HeLa, and U2OS cells were cultured in Dulbecco’s modified Eagle’s medium
and K562 cells in RPMI1640 media that were supplemented with 10% fetal bovine serum and penicillin/streptomycin in an atmosphere of 5% CO₂ at 37 °C. Cell transfection was carried out by liposome-mediated method with lipofectamine reagent (Invitrogen).

**GST Pull-down Assay—** c-Abl or TopBP1 open reading frames were subcloned into pGEX2TK. These fusion proteins were induced with 0.1 mm isopropyl 1-thio-β-D-galactopyranoside in Escherichia coli strain BL21 and purified with GST beads. Cell lysates were prepared and incubated with GST fusion proteins at 4 °C overnight. The precipitates were washed with phosphate-buffered saline and analyzed by SDS-PAGE followed by Coomassie Blue staining. Immunoprecipitation, Western blot analysis, and in vitro kinase assay IP and Western blots were performed as previously reported (30, 31). For kinase assays, c-Abl was immunoprecipitated from MEFs and washed thoroughly with TNEN buffer (plus 0.5% Triton X-100) 5 times followed by washing with the kinase buffer 2 times. GST-TopBP1 was incubated with c-Abl in kinase buffer (20 mM Tris-HCl (pH 7.4), 10 mM MnCl₂, and 10 mM MgCl₂) containing [γ-32P]ATP or unlabelled ATP for 30 min at 30 °C. The reaction products were analyzed by SDS-PAGE and autoradiography.

**Luciferase Assay—** 1.5 kilobases of c-Abl promoter was cloned into pG3L by restriction digestion of Mlu/Hind III. The expression constructs (2 μg of c-Abl, TopBP1, KD-c-Abl), the promoter plasmid (2 μg of pGL3-Luc), and 0.4 μg of Renilla plasmid were co-transfected into HeLa cells as described above. Cells were harvested 1–2 days later and lysed in reporter lysis buffer (Promega). The luciferase activity and Renilla activity were measured following the manufacturer's protocols. The luciferase activity was normalized against the Renilla activity. All assays were carried out in triplicate.

**Morpholino Oligomers and siRNA Inhibition of TopBP1 Expression—** The morpholino oligomer used was AS 5'-TTGGGACACATCGCTCTGGTGGCAT-3'. The control morpholino oligonucleotide was 5'-TTAACCTCAGTTACAATTTATA-3'. The conditions to amplify c-Abl were 35 cycles of 94 °C (30 s), 57 °C (30 s), and 72 °C (1 min) followed by 72 °C for 10 min.

**RESULTS**

**TopBP1 Interacted with c-Abl In Vitro and in Vivo—** The C-terminal portion of c-Abl (containing the kinase, DNA binding, and actin binding domains, designated as fragments 1, 2, and 3) was fused to GST in pGEX2TK vector, and the proper fusion was confirmed by DNA sequencing (Fig. 1A). GST-Abl fusion proteins were expressed in BL21 cells and purified with GST beads. To perform a pull-down experiment, GST-Abl beads were mixed with total cell lysate from a murine osteoblast line MC3T3-E1 and incubated overnight at 4 °C. The beads were then washed five times with phosphate-buffered saline and analyzed on SDS-PAGE gels followed by Coomassie Blue staining. It was found that several proteins were consistently precipitated by GST-Abl but not by GST alone in this repeated pull-down experiments. These protein bands were excised from the gels and sequenced by mass spectrometry. One band was identified as TopBP1 based on sequence homology and the size of the protein. TopBP1 is a 180-kDa protein containing eight BRCT repeats and is implicated in DNA damage-induced cell cycle control and apoptosis (26). To confirm this interaction, three GST-Abl fusion proteins (F1–3) were used to precipitate TopBP1 overexpressed in MC3T3-E1 cells. It was found that fragments 1 and 2, but not fragment 3, were able to precipitate TopBP1, suggesting that the DNA binding domain of c-Abl plays an important role in interacting with TopBP1 (Fig. 1, B and C). Even though the input of F1 fragment in the pull-down experiment was much less than F2, similar amounts of TopBP1 were precipitated, suggesting that the F1 binds to TopBP1 better than F2 and that the kinase domain could also contribute to c-Abl-TopBP1 interaction.

Because c-Abl is able to directly bind DNA and TopBP1 too has the potential for DNA binding, e.g. TopBP1 can bind to DNA double-stranded breaks, it is possible that DNA mediated the interaction between c-Abl and TopBP1. To exclude this possibility, the samples were treated with ethidium bromide to disrupt the double-stranded DNA structure. The interaction between c-Abl and TopBP1 was still observed under this condition (Fig. 1C, lower panel), suggesting a direct interaction.

To confirm this interaction in vivo, co-immunoprecipitation experiments were carried out. The constructs expressing c-Abl, TopBP1 (Myc-tagged), or c-Abl and TopBP1 were transfected into COS7 cells. TopBP1 and associated proteins were immunoprecipitated from the cell lysate using a monoclonal anti-TopBP1 antibody and fractionated on 7.5% SDS-PAGE gels. It was found that c-Abl was in the precipitates brought down by TopBP1 (Fig. 1D). When TopBP1 was not expressed, no c-Abl was detected, suggesting a specific interaction. Reciprocally, TopBP1 was immunoprecipitated with anti-c-Abl antibodies only when c-Abl was co-expressed (Fig. 1E). These results indicate
that c-Abl and TopBP1 do interact in vivo.

We then determined whether the interaction between endogenous c-Abl and TopBP1 existed. TopBP1 was immunoprecipitated with anti-TopBP1 antibodies, and the possibly associated c-Abl was detected by anti-c-Abl antibodies on a Western blot. It was found that c-Abl was present in the precipitates carried down by anti-TopBP1 antibodies but not by control rabbit IgG (Fig. 1F, left panel). Similarly, TopBP1 was found in association with c-Abl (Fig. 1F, right panel). This interaction was observed in several other cell line tested.

TopBP1 Also Interacted with the Kinase Domain of c-Abl and Was an in Vivo Substrate of Abl—We next determined which domain(s) of the c-Abl interacted with TopBP1 by co-immunoprecipitation experiments. Truncated c-Abl fragments were individually co-transfected into COS7 cells with TopBP1 (Fig. 2A). TopBP1 was immunoprecipitated with anti-Myc antibodies. The existence of c-Abl in the complex was detected on a Western blot with anti-c-Abl antibodies (recognizing the SH3 domain at the N' terminus). It was found that c-Abl lacking actin binding domain or lacking both actin binding and DNA binding domains were still able to form a complex with TopBP1. When the kinase domain was further deleted, the interaction became undetectable (Fig. 2B). Note that several truncated c-Abl fragments were observed in co-IP experiments, suggesting that c-Abl is unstable (31). These results suggest that the kinase domain of c-Abl was able to interact with TopBP1. Moreover, the protein level of F5 was much higher than F4, yet similar amounts of F4 and F5 were immunoprecipitated by TopBP1, confirming that the DNA binding domain contributed to c-Abl-TopBP1 interaction (Fig. 1B). Based upon the co-immunoprecipitation and pull-down results, we conclude that both the kinase and the DNA binding domains were able to bind TopBP1 (Figs. 1, A–C, and 2, A and B). Other proteins that interact with the kinase domain of c-Abl include proliferation-associated gene and Rb (4).

Having shown that TopBP1 interacted with the kinase and DNA binding domains of c-Abl, we then tested whether TopBP1 was a substrate for c-Abl. TopBP1 and increasing amounts of c-Abl were transfected into COS7 cells. TopBP1 was immunoprecipitated with anti-TopBP1 antibodies, and its tyrosine phosphorylation was detected by Western blot using anti-phosphotyrosine antibodies. It was found that c-Abl could tyrosine phosphorylate TopBP1 in a dose-dependent manner when co-expressed (Fig. 2C), whereas kinase-dead c-Abl failed to do so (data not shown).

To determine whether TopBP1 is a direct Abl substrate under conditions where Abl is activated, e.g., DNA damage, we treated cells with adriamycin, a chemotherapeutic agent against a wide variety of cancers, and a topoisomerase II inhibitor that has been shown to induce single- and double-stranded DNA breaks (32); an in vitro kinase assay was performed using GST-TopBP1 as a substrate. Serial deletion experiments revealed that the N' portion of TopBP1 (containing the first four BRCT domains) was tyrosine-phosphorylated by c-Abl when co-expressed (data not shown). Endogenous c-Abl was immunoprecipitated from wild type MEFs with the c-Abl−/− MEF as a control (treated or untreated with 1 μM adriamycin for 4 h). GST-TopBP1 was purified and mixed with the beads in the presence of [γ-32P]ATP and then fractionated onto a SDS-PAGE gel, dried, and exposed to x-ray films. It appeared that c-Abl can directly phosphorylate TopBP1 in vitro, and adriamycin treatment increased the kinase activity of c-Abl (Fig. 2D). Auto-phosphorylation of c-Abl was also observed (Fig. 2D). No phosphorylation was observed for c-Abl−/− MEFs.

We then tested the phosphorylation of endogenous TopBP1 by c-Abl. We used MEF, U2OS, HeLa, and MC3T3-E1 cells for the experiments and found that tyrosine phosphorylation of TopBP1 was beyond detection on a Western blot (data not shown). This could be attributed to the fact that neither c-Abl nor TopBP1 was highly expressed in these cells. We then tried K562 cells that are known to carry highly activated BCR-ABL. K562 cells were treated with adriamycin for 1 or 4 h, and endogenous TopBP1 was immunoprecipitated. Tyrosine phosphorylation of TopBP1 was detected by Western blot analysis using anti-phosphotyrosine antibodies. It was found that TopBP1 was indeed phosphorylated upon adriamycin treatment in K562 cells, especially after 1 h of treatment (Fig. 2E). Pretreating cells with a specific inhibitor for Abl kinases, STI571 (1 μM is sufficient to inhibit Abl activity, data not shown), abolished the phosphorylation (Fig. 2E), suggesting the phosphorylation of TopBP1 was carried out by BCR-ABL. Furthermore, it was found that endogenous TopBP1 and BCR-ABL formed a complex under this condition, justified by co-IP experiments (Fig. 2F). These data suggest that BCR-ABL can interact with TopBP1 and phosphorylate TopBP1 upon DNA...
TopBP1 Represses c-Abl Expression

Ectopic expression of TopBP1 down-regulated c-Abl expression. COS7 cells were transfected with 0.01, 0.05, 0.25, or 1 μg of TopBP1 construct for 24 h. The levels of c-Abl and TopBP1 were assessed by Western blot. Time course study of c-Abl repression by TopBP1. COS7 cells were transfected with 2 μg of TopBP1 construct for 6, 12, 24, 32, or 42 h. Mock served as a control in which empty vector was used to transfect the cells. The levels of c-Abl and TopBP1 were assessed by Western blot. The blot was stripped and re-probed with anti-p53, protein kinase Ca (PKCa), or actin as controls. c-Abl expression was similarly inhibited by TopBP1 in HeLa cells. HeLa cells were transfected with TopBP1 construct for 6, 12, 24, 32, and 42 h or with empty vector (M). The levels of c-Abl were assessed by Western blot. D, overexpression of c-Abl did not alter the expression of TopBP1. COS7 cells were transfected with increasing amounts of c-Abl for 24 h. A Western blot was carried out to determine the expression of TopBP1.

damage. This could be achieved by BCR-ABL translocation to the nucleus where TopBP1 is located (17). These results strongly suggest that TopBP1 is a substrate of Abl in response to DNA damage.

Ectopic Expression of TopBP1 Down-regulated Endogenous c-Abl Expression at the Protein Level—During co-immunoprecipitation experiments, we noted that expression of TopBP1 in COS7 cells led to a dramatic reduction in protein levels of endogenous c-Abl. To confirm this observation, we transfected COS7 cells with increasing amounts of c-Abl expressing DNA construct expressing endogenous c-Abl. To test whether a reciprocal relationship existed, we transfected HeLa with different amounts of transfection reagent were transfected into COS7 cells. After 24 h cells were collected, and equal amounts of total cell lysates were analyzed by Western blot for TopBP1 and c-Abl expression. B, overexpression of c-Abl did not alter the expression of TopBP1. COS7 cells were transfected with increasing amounts of c-Abl for 24 h. A Western blot was carried out to determine the expression of TopBP1.

Knocking Down TopBP1 Released c-Abl Repression—We have shown that TopBP1 expression was sufficient to repress c-Abl expression. In fact, a severalfold increase in TopBP1 was able to repress c-Abl expression dramatically (Fig. 3, B and C). To test whether TopBP1 is required for normal expression of c-Abl, we knocked down the expression of TopBP1 using the morpholino antisense oligos (AS) (26). These antisense oligos could significantly lower the protein levels of TopBP1 when transfected into HeLa cells (Fig. 4A). Re-probing the blot with anti-c-Abl antibodies revealed that c-Abl protein levels increased whenever TopBP1 is reduced (Fig. 4A). This inverse correlation strongly suggests that TopBP1 is required to repress c-Abl expression. When TopBP1 protein is lower or absent, the repression is released and, thus, increased c-Abl expression. To further support this conclusion, we repeated this knocking down experiment with siRNA in the presence of TopBP1 overexpression. It was observed that the siRNA was able to dramatically reduce the TopBP1 levels even when it was driven by a cytomegalovirus promoter, whereas the control siRNA did not (Fig. 4B). It was also observed that siRNA eliminated the repression of c-Abl by TopBP1 (Fig. 4B). These results indicate that TopBP1 could repress c-Abl expression at either the basal level or when overexpressed.

TopBP1 Repressed c-Abl Expression at mRNA Levels—To determine whether TopBP1 overexpression would lead to a rapid degradation of c-Abl, which is known to be a substrate of the ubiquitin-proteosome pathway when activated, we treated the TopBP1-transfected cells with a proteasome inhibitor MG132 to inhibit protein degradation. It was found that the addition of MG132 at the start of or 6 h post-transfection alleviated the repression on c-Abl protein level. HeLa cells were transfected with TopBP1 plus the control siRNA or siRNA for TopBP1 (siTopBP1). The protein levels of c-Abl and TopBP1 were assessed by Western blot analysis.

To test whether the regulation occurs at the level of transcription, the levels of c-Abl mRNA were first assessed by RT-PCR. Most human tissues and cell lines express two abundant mRNA species, type 1a (6 kilobases) and 1b (7 kilobases), as well as minor species (33). Primers were designed so that the two major species of c-Abl mRNA could be amplified. HeLa cells were transfected with TopBP1 construct, and total RNA was isolated at different time points. RT-PCR was carried out to determine the mRNA levels of c-Abl with and without TopBP1 expression. We found that ectopic expression of TopBP1 dramatically down-regulated the mRNA levels of c-Abl without
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TopBP1 Repressed c-Abl Expression at the Transcriptional Level

A proteasome inhibitor did not block the down-regulation of c-Abl by TopBP1. COS7 cells were transfected with TopBP1 for 6 h followed by treatment with increasing amounts of MG-132. The levels of c-Abl were assessed by Western blot. TopBP1 overexpression also down-regulated the levels of c-Abl mRNA. COS7 cells were transfected with TopBP1 cDNA for 6, 12, or 24 h or with the empty vector (M). After different periods of time, cells were harvested to analyze the c-Abl mRNA levels by RT-PCR. c-Abl overexpression did not alter the mRNA levels of c-Abl that were driven by cytomegalovirus promoter. COS7 cells were transfected with TopBP1 and increasing amounts of c-Abl for 24 h, and the c-Abl mRNA levels were analyzed by RT-PCR. TopBP1 inhibited the c-Abl promoter activity in a reporter assay. c-Abl expression plasmid (2 µg) was co-transfected with increasing amounts of TopBP1 plasmid. The -fold induction was normalized to the empty vector. Each experiment was carried out in triplicate and was repeated three times. Similar results were obtained.

Roles of Histone Deacetylation and DNA Methylation in Repressing c-Abl Expression—There is no evidence to suggest that TopBP1 binds directly to specific cis elements of its target genes. Therefore, we speculate that an unidentified transcription factor brings TopBP1 to the transcription initiation complex, where TopBP1 might recruit transcription repressors such as Brg1/Brm or HDACs (37). Unlike the E2F target genes, TopBP1 did not require Brg1/Brm to repress c-Abl expression, because TopBP1 similarly repressed the c-Abl expression in C33A cells, a cell line deficient for Brg1/Brm1 (37) (data not shown). On the other hand, using trichostatin A (TSA), an inhibitor for HDACs, we found that histone deacetylation was necessary for TopBP1 to repress c-Abl promoter, because TSA treatment abolished the repression on c-Abl by TopBP1 (Fig. 6, A and B). These results suggest that TopBP1 might repress c-Abl transcription by recruiting HDACs. Consistent with this result, an interaction between TopBP1 and HDAC1, but not HDAC2, -4, and -6, was observed in a co-immunoprecipitation assay (Fig. 6C).

Furthermore, the c-Abl promoter is silenced by hypermethylation in CML myeloid cells (21, 22). To determine whether a link exists between DNA methylation and TopBP1 repression of c-Abl, we studied the effects of TopBP1 overexpression on c-Abl expression in the presence of 5-azacytidine, a DNA methylation inhibitor. It was found that 5-azacytidine treatment up-regulated c-Abl expression in HeLa cells and that ectopic expression of TopBP1 failed to significantly down-regulate c-Abl mRNA levels in the presence of this methylation inhibitor (10 µM) (Fig. 6, D and E). These results taken together suggest...
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assays showing that co-expression of c-Abl but not the kinase-dead c-Abl blocked the repressive effect of TopBP1 on c-Abl promoter activity.

How does TopBP1 repress the expression of c-Abl? Several reports have shown that TopBP1 could act as a transcriptional suppressor. It interacts with E2F and inhibits the expression of E2F target genes by recruiting Brg1/Brm; it also interacts with Miz1 and inhibits the expression of c-Myc (34, 37). Yet TopBP1 repression of c-Abl was not mediated by Miz1 or E2F since neither showed any effect on c-Abl promoter activities. Nor does repression of c-Abl absolutely require Brg1/Brm since it could still occur in Brg1/Brm-deficient C33A cells. Our results instead suggest that TopBP1 might use a novel mechanism to repress c-Abl expression. We propose that TopBP1 might be recruited to the c-Abl promoter by an unidentified transcription factor. TopBP1 may then recruit HDACs and DNA methyltransferases to repress transcription. Several lines of evidence support this hypothesis. First, administration of the histone deacetylase inhibitor TSA blocked the repression on c-Abl promoter by TopBP1. Consistent with our results, HDAC has been found to interact with the BRCT motif (38) and TopBP1 (Fig. 7A).

Another interesting aspect of TopBP1 as a transcriptional repressor is that its target genes are mostly, if not all involved in apoptosis and/or cell cycle control. Although c-Myc is a proto-oncogene, it is also involved in apoptosis induction. E2F1 plays an essential role in S phase entry, and some of its target genes are also pro-apoptotic. Similarly, c-Abl is involved in both cell cycle control and apoptosis. Based upon our findings that c-Abl inhibited the function of TopBP1, we speculate that c-Abl may also regulate other TopBP1 target genes. Indeed c-Myc transcription has been reported to be regulated by c-Abl (42). Whether TopBP1 also mediates this regulation needs further investigation. TopBP1 is required for cell survival, as knocking down of TopBP1 by antisense oligos led to more cell death (26). It is likely that some of its target genes may mediate its pro-survival role. For example, the deficiency of TopBP1 might up-regulate the pro-apoptotic protein such as c-Abl, c-Myc.

What is the significance of interaction between c-Abl and TopBP1? One possibility is to modulate c-Abl expression. c-Abl has to be tightly controlled since elevation in c-Abl levels can induce cell cycle arrest and/or programmed cell death (18, 19). Furthermore, c-Abl levels might fluctuate constantly since it is rapidly degraded once activated (20), e.g. by DNA damage or platelet-derived growth factor, and needs to be replenished. Therefore, cells require a mechanism to maintain a constant level of c-Abl. Based upon our results, we propose that upon activation, c-Abl will be degraded by proteasomes; at the same time, TopBP1, which is constitutively associated with c-Abl, will be phosphorylated, and in turn its repression on c-Abl is relieved, leading to increased c-Abl expression. This feedback regulation can augment c-Abl expression. Still, how cells terminate this amplification is currently unclear. Although

that both histone deacetylation and DNA methylation played a role in TopBP1-induced repression on c-Abl transcription. Co-immunoprecipitation assays did not reveal an evident interaction between endogenous TopBP1 and DNA methyltransferase-1 (data not shown).

c-Abl Negatively Regulated the Transcriptional Repressor Activity of TopBP1—Because c-Abl interacted with and phosphorylated TopBP1, we next tested whether c-Abl has any effect on TopBP1 activity in this reporter assay. Expression of c-Abl itself did not significantly alter the promoter activity of c-Abl (Fig. 7A). However, co-expression of c-Abl and TopBP1 abolished the repressive activity of TopBP1 as the promoter activity returned to the basal level, suggesting that tyrosine phosphorylation by c-Abl interfered with its activity in transcription repression. To prove this a kinase-dead c-Abl was co-expressed with TopBP1 in the reporter assay. It was found that the kinase-dead c-Abl alone did not alter the promoter activity and that it also failed to significantly alter the repressive activity of TopBP1 (Fig. 7A). Because kinase-dead c-Abl interacted with TopBP1 as well as normal c-Abl (Fig. 7B), it is conceivable that tyrosine phosphorylation of TopBP1 by c-Abl might somehow interfere with its transcription repressor activity. The future work is to identify the phosphorylation sites on TopBP1 and test the physiological function of these phosphorylations. This work is complicated by the nature of TopBP1, which contains 8 repeats of BRCT domains that distribute from the N-terminus to the C-terminus.

DISCUSSION

We show here that c-Abl physically interacts with TopBP1 and that TopBP1 is a substrate of Abl proteins. Functionally, these two proteins appeared to display an antagonistic interplay. On the one hand, TopBP1 repressed c-Abl expression at the level of transcription. Overexpression of TopBP1 down-regulated c-Abl expression at both protein and mRNA levels and repressed the promoter activity of c-Abl. Knocking down TopBP1 led to an increase in c-Abl. On the other hand, c-Abl negatively regulated TopBP1 in its repression on c-Abl transcription, which required c-Abl kinase activity, because co-expression of c-Abl but not the kinase dead c-Abl blocked the repressive effect of TopBP1 on c-Abl promoter activity.
TopBP1 was found to repress the expression of c-Abl, another BRCT-containing protein Brca1 was found to inhibit the kinase activity of c-Abl (14). Hence, it appears that c-Abl action can be kept in check by at least two BRCT-containing proteins involved in DNA damage response.

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