The Tandem BRCT Domains of Ect2 Are Required for Both Negative and Positive Regulation of Ect2 in Cytokinesis*

Received for publication, August 13, 2004, and in revised form, October 29, 2004
Published, JBC Papers in Press, November 15, 2004, DOI 10.1074/jbc.M409298200

Ja-Eun Kim, Daniel D. Billadeau, and Junjie Chen‡
From the Department of Oncology Research, Mayo Clinic College of Medicine, Rochester, Minnesota 55905

Epithelial cell transforming protein 2 (Ect2) is a guanine nucleotide exchange factor (GEF) for Rho GTPases, molecular switches essential for the control of cytokinesis in mammalian cells. Aside from the canonical DbI homology/pleckstrin homology cassette found in virtually all Dbl family members, Ect2 contains N-terminal tandem BRCT domains. In this study, we address the role of the Ect2 BRCT domains in the regulation of Ect2 activity and cytokinesis. First, we show that the depletion of endogenous Ect2 by small interfering RNA induces multinucleation, suggesting that Ect2 is required for cytokinesis. In addition, we provide evidence that Ect2 normally exists in an inactive conformation, which is at least partially due to an intramolecular interaction between the BRCT domains and the C-terminal domain of Ect2. This intramolecular interaction masks the catalytic domain responsible for guanine nucleotide exchange toward RhoA. Consistent with a role in regulating Ect2 GEF activity, overexpression of an N-terminal Ect2 containing the tandem BRCT domains, but not single BRCT domain or BRCT domain mutant, leads to a failure in cytokinesis. Surprisingly, although ectopically expressed wild-type Ect2 rescues the multinucleation resulting from the depletion of endogenous Ect2, expression of a BRCT mutant of Ect2 failed to restore proper cytokinesis in these cells. Taken together, the results of our study indicate that the tandem BRCT domains of Ect2 play dual roles in the regulation of Ect2. Whereas these domains negatively regulate Ect2 GEF activity in interphase cells, they are also required for the proper function of Ect2 during cytokinesis.

The small Rho GTPase is known to play important roles in essential cellular processes such as the regulation of actin cytoskeleton, gene transcription, cell motility, cell adhesion, and cytokinesis (1, 2). Rho GTPases function as molecular switches cycling between an inactive GDP-bound state and an active GTP-bound state. This cycle is controlled by several regulatory proteins including guanine nucleotide exchange factors (GEFs)¹ that facilitate the release of GDP from Rho GTPase, GTPase-activating proteins that catalyze GTP hydrolysis, and guanine nucleotide dissociation inhibitors that sequester Rho GTPases in the cytosol and thereby inhibit guanine nucleotide exchange (3).

Of the two families of Rho GEFs, the Dbl domain-containing family is by far the larger one, with over 60 members identified in the human genome (4). These Dbl GEFs contain a common catalytic domain designated the Dbl homology (DH) domain. In addition to the DH domain, most Dbl family members also contain a distal pleckstrin homology (PH) domain that is necessary for their GEF activities (5, 6). Ect2 is a Dbl family member that possesses a DH/PH cassette and mediates the guanine nucleotide exchange of Rho GTPases (7). It was initially identified as a proto-oncogene able to induce transformation when transfected in epithelial cells (8).

Similar to other Dbl family proteins, the transforming activity of Ect2 was the result of an N-terminal deletion (9, 10), implying that the N terminus of Ect2 may serve as a negative regulator of GEF function. The N terminus of Ect2 contains tandem repeats of the BRCT domain, a motif that was first described at the C terminus of the breast cancer susceptibility gene product, BRCA1, and is conserved in many proteins involved in cell cycle checkpoint and DNA damage response (11). It is not yet known how these tandem BRCT repeats regulate Ect2 activity in vivo.

Previous studies have suggested an important role of Ect2 in the regulation of cytokinesis, the last step of cell division partitioning the cytoplasm and the cell cortex to form two daughter cells (12, 13). Pebble, the Drosophila homolog of Ect2, is required for cytokinesis through regulation of a Rho1-dependent signaling pathway (14, 15). In addition, overexpression of human Ect2 N terminus, which contains the tandem BRCT motifs but not the DH/PH domain, leads to multinucleation (7, 16, 17), implying a negative role of the BRCT domains in the regulation of Ect2 activity in vivo.

In this study, we examined the regulatory roles of the Ect2 BRCT domains. We identified an autoinhibitory interaction between the BRCT and DH/PH domains of the Ect2 protein. The release of this autoinhibitory interaction is imperative for the binding and activation of Rho GTPase. Interestingly, the Ect2 BRCT domains also play a positive role and are required for proper cytokinesis in vivo.

EXPERIMENTAL PROCEDURES

Constructs—Human Ect2 cDNA was kindly provided by Dr. T. Miki (National Cancer Institute, Bethesda, MD). FLAG-tagged full-length and deletion fragments were subcloned into pIREs-EGFP mammalian expression vector. The BRCT W210R and W304R mutants were created with the QuikChange site-directed mutagenesis kit (Stratagene). pcDNA3-based full-length and C terminus of Ect2 constructs were generated for in vitro translation studies. Wild-type or mutant Ect2 expression RNA; HA, hemagglutinin; EGFP, enhanced green fluorescent protein; Ect2, epithelial cell transforming protein 2.

¹ The abbreviations used are: GEF, guanine nucleotide exchange factor; DH, Dbl homology; PH, pleckstrin homology; GST, glutathione S-transferase; PBS, phosphate-buffered saline; siRNA, small interfering RNA; HA, hemagglutinin; EGFP, enhanced green fluorescent protein; Ect2, epithelial cell transforming protein 2.
fragments containing residues 1–341 were subcloned into pGEX 4T-1 vector for the expression of glutathione S-transferase (GST) fusion proteins in Escherichia coli. GST-fused RhoA and GST-RhoA N-19 constructs were kindly provided by Dr. D. B. Billadeau (Mayo Clinic, Rochester, MN).

Purification of Recombinant Proteins in E. coli — E. coli expressing the indicated GST fusion proteins were suspended in phosphate-buffered saline (PBS) containing 2% Triton X-100 and lysed by sonication. Cell debris was then pelleted by centrifugation, and the remaining supernatant was incubated with glutathione 4B-Sepharose beads (Amersham Biosciences) at 4 °C for 4 h with rotation. The beads were pelleted and washed with NETN buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, and 0.5% Nonidet P-40). GST fusion proteins were eluted with 100 mM Tris-HCl, pH 8.8, buffer containing 20 mM glutathione and dialyzed against PBS. The integrity of purified proteins was estimated by Coomassie Blue staining after SDS-PAGE.

Antibodies — Rabbit anti-Ect2 antibody was raised by Immunizing rabbits with GST-Ect2 containing amino acids 112–341. Anti-Ect2 antibody was affinity-purified using the AminoLink Plus Immobilization and Purification Kit (Pierce). Anti-RhoA and anti-GST antibody were purchased from Santa Cruz Biotechnology, anti-HA antibody from Covance, and anti-FLAG, anti-α-tubulin, and anti-actin antibody were obtained from Sigma.

Small Interfering RNA — E. coli small interfering RNA (siRNA) was synthesized by Dharmacon Inc. The siRNA duplexes were 21 base pairs synthesized by Dharmacon Inc. The siRNA duplexes were performed using Oligofectamine reagent as described by the manufacturer (Invitrogen).

Cell Culture, Transfection, Immunoprecipitation, and Immunoblotting — All cell lines were obtained from American Type Culture Collection (ATCC) and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum at 37 °C in 5% CO2 (v/v). Cell debris was then pelleted by centrifugation, and the remaining supernatant was washed with NETN buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, and 10 mM MgCl2) was added, and the samples were filtered through a nitrocellulose membrane (Millipore), which was subjected to extensive washing with wash buffer. The radioactivity that remained on the filter was quantified by a liquid scintillation counter.

RESULTS

Ect2 Is Involved in Cytokinesis — Previous studies have implicated a role for Ect2 in transformation and the regulation of cell division. In fact, whereas overexpression of the C-terminal portion of Ect2 containing the DH/PH cassette leads to transformation of NIH 3T3 cells (9, 10), overexpression of the N-terminal region containing the BRCT domains resulted in multinucleation in U2OS and HeLa cells (7, 16, 17). These findings indicate that Ect2 may act as a critical regulator of cell cycle progression. To gain insight into the role of endogenous Ect2 in cell cycle regulation, we depleted Ect2 using siRNA. Ect2 expression was significantly down-regulated in HeLa cells by ect2 siRNA (Fig. 1A). We monitored cell morphology in Ect2 siRNA-transfected cells by immunofluorescence staining with anti-Ect2 and anti-actin antibodies (Fig. 1B). As shown in Fig. 1C, the number of multinucleated cells substantially increased in Ect2 siRNA-transfected cells, demonstrating that Ect2 is required for cytokinesis.

BRCT Domain of Ect2 Is Responsible for Intramolecular Interaction between the N Terminal and C Terminal of Ect2, but not for Intramolecular Oligomerization of Ect2 — Ect2 is a Rho GEF whose exchange activity increases in mitosis, coincident with its hyperphosphorylation in M phase (7). However, it is not clear mechanistically how the regulation of Ect2 GEF activity is achieved. Many of the well-characterized Dbl family members exist in an inactive or partially active state through intramolecular or intermolecular interactions that are subsequently released after stimulation (4, 19). The previous observations that overexpression of either the N or C terminus of Ect2 showed different effects on cell cycle regulation suggested a potential mechanism of autoinhibition of Ect2 activity through intramolecular interaction. To test this hypothesis, we employed a GST pull-down assay using the bacterially expressed GST-Ect2-N construct containing the tandem BRCT domains to detect a potential interaction with the C-terminal half of the Ect2 molecule, which was translated in vitro. Consistent with our hypothesis, GST-Ect2-N spanning amino acids 1–341 interacted with full-length Ect2 (F; amino acids 1–882) and the C terminus of Ect2 (C; amino acids 342–882), but not with Ect2-N itself (N) (Fig. 2A), whereas the GST control did not bind to any of the three fragments. This indicates that the N terminus of Ect2 physically interacts with the C terminus of Ect2 that contains the DH/PH module.

Next, we sought to determine whether the intact structure of the BRCT domains at the N terminus of Ect2 is required for this intramolecular interaction. To answer this question, a GST

Na3VO4) at 4 °C for 20 min. The beads were washed three times with binding buffer containing 1% Nonidet-P40, and the bound material was subjected to SDS-PAGE and Western blotting.

In Vitro GDP/GTP Exchange Assays — GDP/GTP exchange assays were performed as described previously (18). Briefly, 293T cells transiently transfected with FLAG-tagged expression constructs encoding the full length, BRCT mutant, or C terminus of Ect2 were lysed in cold lysis buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, and 0.5% Nonidet P-40). Anti-FLAG M2-agarose (Sigma) was added to cell lysate, followed by incubation at 4 °C for 1 h with gentle mixing. Immunoprecipitates were washed twice with lysis buffer and twice with wash buffer and subjected to GDP/GTP exchange assays. The RhoA-[3H]GDP complex was prepared by incubation of RhoA with 0.5 μM [3H]GDP (Amersham Biosciences) in exchange buffer (20 mM Tris-HCl, pH 8.0, 3 mM MgCl2, 10 mM EDTA, and 1 mM dithiothreitol) at 37 °C for 20 min followed by the addition of 8 mM MgCl2 to terminate the GDP/GTP exchange reaction. Immunoprecipitates were incubated with the RhoA-[3H]GDP complex at room temperature with 1 μM GTP. After incubation for specified periods, ice-cold wash buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, and 10 mM MgCl2) was added, and the samples were filtered through a nitrocellulose membrane (Millipore), which was subjected to extensive washing with wash buffer. The radioactivity that remained on the filter was quantified by a liquid scintillation counter.
fusion protein of Ect2 encompassing only the tandem BRCT repeats (amino acids 112–341) was used for the pull-down assay as described above. This shortened N terminus of Ect2 also showed a strong interaction with the C terminus of Ect2 (data not shown). To confirm that the BRCT domain mediates this intramolecular interaction, a point mutation (W304R) was introduced into the BRCT domain by replacing a highly conserved tryptophan with arginine in the α3 helix of BRCT domain (11) that is involved in the folding of BRCT domains (20).

Unlike wild-type GST-Ect2-N, the GST-Ect2-N-W304R mutant was unable to interact with either full-length or C-terminal Ect2 protein (Fig. 2A), indicating that the intact BRCT domains of Ect2 are critical for this intramolecular interaction. The other regulatory mode of Dbl family members involves oligomerization through an intermolecular interaction, as suggested by studies of RasGRF, Dbl, Lsc, and p115RhoGEF (21–24). We tested the potential oligomerization of Ect2 by transient co-transfection of 293T cells with FLAG- and HA-tagged full-length Ect2 constructs. Immunoprecipitation with antibodies recognizing the FLAG epitope showed a specific co-precipitation of HA-tagged Ect2, demonstrating that Ect2 can exist as oligomers in vivo (Fig. 2B). We next examined whether this homo-oligomerization of Ect2 is dependent on the BRCT domain of Ect2. As shown in Fig. 2B, the BRCT mutant of Ect2 (F-W304R) still has the ability to oligomerize in vivo. As a whole, these data indicate that the BRCT domain is required for the intramolecular interaction, but not for the intermolecular oligomerization of Ect2.

The above-characterized intramolecular interaction mediated by the BRCT domain between the N terminus and C terminus of Ect2 raised the possibility that the release of this interaction might lead to the activation of Ect2 by exposing the catalytic domain of Ect2 to a Rho GTPase. RhoA, Rac1, and Cdc42 have all been suggested as substrates of Ect2 in vitro and in vivo (7, 10). However, the Drosophila Ect2 homolog Pebble interacts only with Rho1, and not with Rac1 or Cdc42 (15). Moreover, siRNAs targeting pbl or Rho1 lead to multinucleation, whereas siRNA targeting Rac1 or Cdc42 does not show such a phenotype (25). In addition, a recent study demonstrated the localization of RhoA, Rac1, and Cdc42 during cytokinesis (26). Rac1 was aligned along the central spindle, and Cdc42 seemed to be encircled by the equatorial ring, whereas only RhoA was positioned in the midbody around the equatorial ring, a pattern similar to that of Ect2. To determine whether RhoA is associated with Ect2 during cytokinesis in vivo, we compared the
localization pattern of RhoA with that of Ect2 during cytokinesis by morphologically examining the staining pattern of Ect2 and RhoA with anti-H9251-tubulin staining that serves as a midbody marker. Indeed, Ect2 co-localized with RhoA at the midbody (Fig. 3A).

Based on these data, we focused on RhoA and tested whether or not the BRCT mutant of Ect2, which loses an intramolecular interaction, should allow the catalytic domain of Ect2 to open and interact more strongly with a dominant negative RhoA, RhoA-N19. GST or GST-RhoA N-19 was incubated with cell lysates prepared from 293T cells that were transfected with either the full-length construct (F), the BRCT domain mutant (F-W304R), or a truncation mutant containing the DH/PH cassette (C). The Ect2-C containing the catalytic domain formed a strong complex with GST-RhoA-N19, whereas the wild-type Ect2-F did not bind RhoA in this assay. However, Ect2-F-W304R demonstrated increased interaction with RhoA (Fig. 3B). This supports our model that BRCT mutation abolishes the intramolecular interaction between the N terminus and C terminus of Ect2 (Fig. 2A) and thus allows the binding of the catalytic domain with RhoA (Fig. 3B).

To further confirm that the release of the intramolecular interaction results in increased GEF activity of Ect2, we examined the biochemical activity of wild-type and mutant Ect2 to catalyze the exchange of GDP to GTP in vitro. GST-RhoA was loaded with [3H]GDP and subsequently incubated with immunoprecipitated complexes from 293T cells expressing the wild-type (F), the BRCT domain mutant (F-W304R), or the constitutively active version of Ect2 (C). As shown in Fig. 3C, the release of GDP was measured over time. The constitutively active Ect2-C significantly catalyzed the release of GDP from RhoA over the time course examined. In contrast, the wild-type Ect2 did not demonstrate any detectable GEF activity, whereas the Ect2-F-W304R mutant exerted increased GEF activity. These results again support that the release of the intramolecular interaction imposed by the BRCT domain exposes the Ect2 catalytic domain and thus increases the GEF activity of Ect2 toward RhoA.

The Tandem BRCT Domains Are Responsible for the Multinucleation Phenotype Observed in Cells Overexpressing the N Terminus of Ect2—Our data suggest that the BRCT domains of Ect2 participate in the negative regulation of Ect2 GEF activity in part through a mechanism of autoinhibition. Next, we sought to clarify the biological role of the Ect2 BRCT domains and their potential role in the regulation of cytokinesis in vivo. HeLa cells were transiently transfected with plasmids encoding wild-type or various mutants of FLAG-tagged, EGFP-tagged Ect2 (Fig. 4A). Expression of wild-type or mutant Ect2 was confirmed by Western blotting using anti-FLAG antibody (Fig. 4B). 72 h after transfection, multinucleated cells were determined in EGFP-positive transfectants (Fig. 4C). We confirmed that overexpression of the N terminus of Ect2 containing the tandem BRCT domains (N2) led to multinucleation, whereas these phenotypic changes were not observed in cells transfected with vector alone, or in cells overexpressing either full-length Ect2 (F) or the C terminus of Ect2 (C) (Fig. 4D). Interestingly, the multinucleation phenotype induced by overexpression of the Ect2-N2 fragment was totally abolished by introduction of the W304R mutation in the N2 fragment, demonstrating that the intact BRCT domain is required for...
multinucleation phenotype (Fig. 4D). Notably, the overexpression of N-terminal Ect2 without BRCT II domain (N1) also showed no defect in cytokinesis (Fig. 4D). From these data, we conclude that the ability of the N terminus of Ect2 to induce the multinucleated phenotype depends on the tandem BRCT domains.

**The BRCT Domain Is Essential for Ect2 Biological Activity during Cytokinesis**—Our findings suggest that the tandem BRCT domains of Ect2 function as a single unit in controlling cytokinesis at least in part through negative regulation of the Ect2 GEF activity. If the tandem BRCT domains of Ect2 are involved only in the negative regulation, we would expect that these domains would be dispensable for Ect2 activity in cytokinesis in vivo. To test this, we transfected HeLa cells with a vector control or siRNA-resistant versions of the wild-type (F) or BRCT mutant of Ect2 (F-W304R). We subsequently transfected these cells with Ect2 siRNA and then checked the expression level of endogenous and exogenous Ect2 by either anti-Ect2 antibody (data not shown) or anti-FLAG antibody (Fig. 5A). The endogenous Ect2 was completely depleted by siRNA transfection. However, siRNA-resistant Ect2-F and Ect2-F-W304R were still expressed in these cells. We quantified the percentages of multinucleated cells in each transfection. Similar to that shown above in Fig. 1C, depletion of Ect2 results in a substantial increase of multinucleated cells (Fig. 5B). Strikingly, although the multinucleated phenotype could be rescued by the expression of wild-type Ect2, the BRCT domain mutant failed to restore normal cytokinesis (Fig. 5B). These data strongly suggest that the tandem BRCT domains are not only involved in negatively regulating Ect2 GEF activity but also are essential for the biological activity of Ect2 in cytokinesis.

**DISCUSSION**

The present study demonstrates that the tandem BRCT domains of Ect2 play an essential role in regulating the biochemical and biological activities of Ect2. Ect2 is required for proper cytokinesis, as evidenced by the high level of multinucleation in cells treated with Ect2 siRNA (Fig. 1). The N-terminal BRCT domains mediate an intramolecular interaction between the N terminus and C terminus of Ect2 (Fig. 2A) but are dispensable for the homo-oligomerization of Ect2 (Fig. 2B). Whereas the BRCT domains play an autoinhibitory role in the regulation of Ect2 GEF activity toward RhoA (Fig. 3), they are also required for proper cytokinesis (Fig. 5). Overall, these findings suggest that the tandem BRCT domains play both negative and positive roles in regulating Ect2 activity in vivo.

Many of the Dbl family members are regulated through a negative intramolecular interaction that is relieved after specific signaling events resulting in phosphorylation (e.g., Vav and proto-Dbl) or protein binding (e.g., Asef and p115RhoGEF) (27–31). The GEF activity of Ect2 is regulated by a similar mechanism. As shown in our study, the tandem BRCT domains of Ect2 block the catalytic domain of Ect2 through an intramolecular interaction. Indeed, Ect2 protein immunoprecipitated from unsynchronized cells, in which most of the cells are in interphase, shows little GEF activity, suggesting that Ect2 exists mainly in an inactive oligomeric form during interphase.
In mitosis, Ect2 is activated and required for cytokinesis. However, little is known about how Ect2 is activated during mitosis. Although the phosphorylation of Ect2 in mitosis correlates with the increased GEF activity of Ect2, it is not yet known whether Ect2 phosphorylation is the cause or the consequence of conformational changes leading to the increased GEF activity. Similar to Asef or p115RhoGEF, the biological activity of Ect2 may also be controlled through the recruitment of additional protein(s) that binds directly to Ect2 and thus relieves its intramolecular autoinhibition (Fig. 6). Future studies are needed to further elucidate the mechanism by which the intramolecular inhibition of Ect2 is relieved during mitosis.

Ect2 activity may also be regulated by intermolecular interaction through Ect2 oligomerization. For example, oligomerization of RasGRF1 and RasGRF2 through the DH domain (21), oligomerization of Dbl through the DH domain (22), oligomerization of Lsc through the coiled-coil domain in the C terminus of Lsc (24), and oligomerization of p115RhoGEF through the C-terminal tail of p115RhoGEF (23) have been reported previously. Oligomerization of these GEFs is important for augmenting the activation of GTPase by recruiting multiple Rho GTPases into the same signaling complex. We have shown here that Ect2 can also form oligomers. However, unlike the intramolecular interaction, the Ect2 BRCT domain is not required for this intermolecular oligomerization. It remains to be determined whether the oligomerization of Ect2 is regulated and whether this property of Ect2 is also essential for the function of Ect2 in cytokinesis.

The activated Ect2 catalyzes the exchange of GDP to GTP and thus activates Rho GTPase during mitosis. However, this study indicates that the increase of Ect2 GEF activity alone is not sufficient for the proper completion of cytokinesis. This is based on our observation that a full-length Ect2 BRCT domain mutant, which still retains GEF activity in vitro and can oligomerize in vivo, failed to rescue the multinucleation phenotype in Ect2 siRNA-treated cells. We propose that the tandem

![Fig. 5. The BRCT mutant of full-length Ect2 fails to restore normal cytokinesis in Ect2 siRNA-transfected cells. HeLa cells were transfected with FLAG-Ect2-pIRES-EGFP constructs (F) that are resistant to Ect2 siRNA. 4 h after recovery from the first transfection, cells were transfected twice with Ect2 siRNA to inhibit the expression of endogenous Ect2. A, the expression levels of siRNA-resistant wild-type and mutant Ect2 were determined by anti-FLAG immunoblotting. B, the percentages of multinucleated cells were determined using fluorescence microscope.](http://www.jbc.org/content/5738)

![Fig. 6. A model of cell cycle-dependent regulation of Ect2. The tandem BRCT domains of Ect2 negatively inhibit its catalytic activity in interphase cells. After the breakdown of nuclear membrane in mitosis, the same BRCT domains may act as a positive regulatory motif by mediating the binding of signaling protein(s) that is necessary for the completion of cytokinesis.](http://www.jbc.org/content/5738)

Regulatory Role of Ect2 BRCT Domains in Cytokinesis

- [Fig. 6](http://www.jbc.org/content/5738)
- [Fig. 5](http://www.jbc.org/content/5738)
BRCT domains may bind additional partner that is prerequisite for the faithful completion of cytokinesis (Fig. 6). We have shown that overexpression of the N terminus of Ect2 has a dominant negative effect in cytokinesis. This effect may be partially due to the exogenous Ect2 BRCT domains competing with endogenous Ect2 for the binding of the Ect2 BRCT domain-binding partner that is needed for cytokinesis. Therefore, the identification of such Ect2 BRCT domain-binding partner(s) will be critical for understanding of the role of Ect2 in the regulation of cytokinesis.

Acknowledgments—We are grateful to Dr. Michael J. Hamann for providing constructs and helpful discussion. We also thank members of the Chen laboratory for discussion and Katherine Minter-Dykhouse, Jamie Wood, and Irene M. Ward for proofreading the manuscript.

REFERENCES
1. Etienne-Manneville, S., and Hall, A. (2002) Nature 420, 629–635
2. Kaibuchi, K., Kuroda, S., and Amano, M. (1999) Annu. Rev. Biochem. 68, 459–486
3. Narumiya, S. (1996) J. Biochem. (Tokyo) 120, 215–228
4. Schmidt, A., and Hall, A. (2002) Genes Dev. 16, 1587–1609
5. Erickson, J. W., and Cerione, R. A. (2001) Biochemistry 40, 837–842
6. Hoffman, G. R., and Cerione, R. A. (2002) Genes Dev. 16, 1587–1609
7. Tatsumoto, T., Xie, X., Blumenthal, R., Okamato, I., and Miki, T. (1999) J. Cell Biol. 147, 921–928
8. Miki, T., Smith, C. L., Long, J. E., Eva, A., and Fleming, T. P. (1993) Nature 362, 462–465
9. Saito, S., Liu, X. F., Kamijo, K., Raziuddin, R., Tatsumoto, T., Okamato, I., Chen, X., Lee, C. C., Lorenzi, M. V., Ohara, N., and Miki, T. (2004) J. Biol. Chem. 279, 7169–7179
10. Solski, P. A., Wilder, R. S., Rossman, K. L., Sondek, J., Cox, A. D., Campbell, S. L., and Der, C. J. (2004) J. Biol. Chem. 279, 25226–25233
11. Callebaut, I., and Murnon, J. P. (1997) FEBS Lett. 400, 25–30
12. Glotzer, M. (2001) Annu. Rev. Cell Dev. Biol. 17, 351–388
13. Maddox, A. S., and Oegema, K. (2003) Mol. Cell 11, 846–849
14. Somma, M. P., Fasulo, B., Cenci, G., Cundari, E., and Gatti, M. (2002) Mol. Biol. Cell 13, 2448–2460
15. Prokopenko, S. N., Brumby, A., O’Keefe, L., Prior, L., He, Y., Saint, R., and Bellen, H. J. (1999) Genes Dev. 13, 2301–2314
16. Kimura, K., Tsuji, T., Takada, Y., Miki, T., and Narumiya, S. (2000) J. Biol. Chem. 275, 17233–17236
17. Saito, S., Tatsumoto, T., Lorenzi, M. V., Chedid, M., Kapoor, V., Sakata, H., Rubin, J., and Miki, T. (2003) J. Cell. Biochem. 90, 819–836
18. Kiyono, M., Satoh, T., and Kaziro, Y. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4826–4831
19. Zheng, Y. (2001) Trends Biochem. Sci. 26, 724–732
20. Zhang, X., Morera, S., Bates, P. A., Whitehead, P. C., Coffer, A. I., Hainbucher, K., Nash, R. A., Sternberg, M. J., Lindahl, T., and Freemont, P. S. (1998) EMBO J. 17, 6404–6411
21. Anborgh, P. H., Qian, X., Papageorge, A. G., Vass, W. C., DeClue, J. E., and Lowy, D. R. (1999) Mol. Cell. Biol. 19, 4611–4623
22. Zhu, K., Debreceni, B., Bi, F., and Zheng, Y. (2001) Mol. Cell. Biol. 21, 425–437
23. Chikumi, H., Barac, A., Bebhahani, B., Gao, Y., Teramoto, H., Zheng, Y., and Gutkind, J. S. (2004) Oncogene 23, 233–240
24. Eisenhaure, T. M., Francis, S. A., Willison, L. D., Coughlin, S. R., and Lerner, D. J. (2003) J. Biol. Chem. 278, 39075–39084
25. Somers, W. G., and Saint, R. (2003) Dev. Cell 4, 29–39
26. Minoshima, Y., Kawashima, T., Hirase, K., Tomozuka, Y., Kawajiri, A., Bao, Y. C., Deng, X., Tatsuka, M., Narumiya, S., May, W. S., Jr., Nosaka, T., Semba, K., Inoue, T., Satoh, T., Inagaki, M., and Kitamura, T. (2003) Dev. Cell 4, 549–560
27. Aghazadeh, B., Lowry, W. E., Huang, X. Y., and Rosen, M. K. (2000) Cell 102, 625–633
28. Bi, F., Debreceni, B., Zhu, K., Salani, B., Eva, A., and Zheng, Y. (2001) Mol. Cell. Biol. 21, 1463–1474
29. Hart, M. J., Jiang, X., Kozasa, T., Roscoe, W., Singer, W. D., Gilman, A. G., Sternweis, P. C., and Bollag, G. (1998) Science 280, 2112–2114
30. Kawasaki, Y., Senda, T., Ishidate, T., Koyama, R., Morishita, T., Iwayama, Y., Hipachi, O., and Akiyama, T. (2000) Science 289, 1184–1187
31. Movilla, N., and Bustelo, X. R. (1999) Mol. Cell. Biol. 19, 7870–7885
The Tandem BRCT Domains of Ect2 Are Required for Both Negative and Positive Regulation of Ect2 in Cytokinesis
Ja-Eun Kim, Daniel D. Billadeau and Junjie Chen

J. Biol. Chem. 2005, 280:5733-5739.
doi: 10.1074/jbc.M409298200 originally published online November 15, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M409298200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 31 references, 16 of which can be accessed free at http://www.jbc.org/content/280/7/5733.full.html#ref-list-1