Survivin is a member of the "inhibitor-of-apoptosis" protein family, defined in part by the presence of a zinc-binding BIR domain. Most BIR domains bind short sequences beginning with alanine, and in this manner they recognize and block the action of key targets in apoptotic pathways. However, Survivin binds only very weakly to typical IAP ligands. Unique features of Survivin are the long C-terminal helix following the BIR domain and a short segment (linking the helix and BIR domains) that mediates Survivin homodimerization. Despite this detailed knowledge of the structure of Survivin itself, there is a current lack of understanding about how Survivin recognizes cellular binding partners, and consequently, many unanswered questions about Survivin function. We determined two co-crystal structures of Survivin and a minimal binding fragment from the chromosomal passenger protein Borealin, a well-validated functional interactor. The interaction between Survivin and Borealin involves extensive packing between the long C-terminal helix of Survivin and a long Borealin helix. Surprisingly, an additional important interaction occurs between the Survivin homodimerization interface and a short segment of Borealin. This segment both structurally mimics and displaces one Survivin monomer. The relevance of this unexpected interaction was tested by mutagenesis of two key Borealin residues. Mutant Borealin introduced into HeLa cells failed to localize properly during mitosis and also caused mislocalization of other chromosomal passenger proteins. This suggests that the mutant is dominant negative and confirms the functional importance of the interaction surface identified in the crystal structures.

In the ten years since Survivin was discovered (1), it has seemed at times to be a protein with too many functions. Survivin is a member of the baculovirus IAP-repeat (BIR2)-domain family. Originally described as an inhibitor-of-apoptosis (IAP) protein, it now assumes an increasingly complex role in the intrinsic cell death pathway (2). Survivin has a more clearly understood function in cell division (3). Despite intensive efforts to define Survivin function, little is known about how Survivin recognizes its binding partners. Indeed, the only three-dimensional structures available are for Survivin itself (4–7). Crystallized Survivin forms a distinctive "bow tie-shaped" (4,5) homodimer. Stable solution dimerization occurs through the same interface (7), suggesting that this dimer may contribute to Survivin function.

Throughout mitosis, Survivin is present in the chromosomal passenger complex (CPC), where it is critically important for mitotic progression (8,9). The CPC is currently known to have three members in addition to Survivin: the kinase Aurora B, an adapter protein INCENP, and a recently discovered addition, Borealin (10–12). A key property of the CPC is that all members are obligate. Depletion of any one member causes mislocalization of all of the others and causes a variety of mitotic abnormalities, consistent with loss of complex function. Early in mitosis, the CPC is found along chromosome arms, but by prophase, it has concentrated at the inner centromeres. It is not clear why the CPC concentrates at the centromeres, as no direct receptor has been identified. One recent candidate is the structural component CENP-C (13), although direct binding to centromeric DNA has also been proposed (14). While at the centromeres, the CPC plays one of its best known roles as part of the spindle assembly checkpoint (SAC). Loss of SAC function leads to chromosome segregation errors and a failure of the cell cycle to arrest in response to spindle poisons. Once all chromosomes have achieved biorientation and are
under tension between the spindle poles, the SAC no longer signals, and the cell enters anaphase. At this time the CPC relocates once more away from the centromeres to the central spindle. As the cell reaches telophase, the CPC concentrates at the midbody where it is essential for cytokinesis. Loss of CPC function at this stage causes a failure of cells to divide fully, leading to binucleate and, eventually, multinucleate cells.

A major structural role of the CPC is to target Aurora B kinase activity to its substrates at appropriate mitotic stages (3,8,9). Because of the mutual functional interdependence of the subunits at all mitotic stages, it has been difficult to dissect how this is achieved. It has been known for some time that the most important direct interaction of CPC members with Aurora B occurs through the short, highly conserved C-terminus of INCENP, called the IN-box. This interaction activates the kinase, and the structure of this subcomplex of the CPC was recently determined (15). In contrast, the extreme N-terminus of INCENP is important for Survivin binding. The discovery of the fourth passenger Borealin has greatly clarified some organizational aspects of this part of the CPC (10–12,14,16,17). Significantly, it was observed by researchers working in different organisms that Borealin and Survivin form a robust binary complex in vitro (10,11). It was further determined that this binary complex has enhanced affinity for INCENP compared to either protein alone (14,16).

We decided to map the interacting regions in the binary complex of the human proteins to better understand the ligand recognition properties of Survivin and to gain insight into CPC organization and function. Through sequence analysis and truncation studies, we identified a minimal fragment of Borealin capable of high-affinity complex formation with Survivin. Surprisingly, crystal structures of two different complexes suggest that the accepted Survivin homodimer is not relevant to mitotic function. Instead, mutation of two key Borealin residues present in its interface with Survivin caused extensive mislocalization of CPC proteins, supporting the functional importance of the new binding interface observed in our structures.

**EXPERIMENTAL PROCEDURES**

*Construction of plasmids for bacterial coexpression.* The gene for full-length human Survivin was subcloned in frame with the N-terminal his$_T$ tag into the T7 expression vector pET-28 (kan$^R$, EMD Biosciences). The gene for human Borealin was instead subcloned into pET21(amp$^R$). A C-terminal Strep-tag (IBA) was added to the Borealin construct by Kunkel mutagenesis. An alternative N-terminal tag (GB1 domain of Protein G) was added to Borealin for some large-scale purifications for crystallography (see below). Truncation mutants were prepared from these constructs by Kunkel mutagenesis. DNA sequences of all inserts were fully verified.

*Coexpression and copurification studies.* The appropriate Survivin and Borealin constructs were cotransfected into BL21–DE3 pLysS Rosetta 2 (EMD Biosciences). Positive colonies were selected on 50 µg/mL kanamycin and 50 µg/mL carbenicillin. Single colonies were grown in LB broth at 37 °C under antibiotic selection until culture density reached $A_{600}$ of 0.6. Expression was induced by addition of 0.4 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG), and the cultures were shaken overnight at 22 °C. Harvested cells were lysed in 100 mL Buffer A (150 mM Tris, pH 7.5; 250 mM NaCl; 0.1 mM TCEP) per liter of culture by passage through a microfluidizer, and the soluble lysate was loaded onto a Ni-NTA agarose column (Qiagen; 0.5 mL resin/liter of culture). The column was washed with 20 volumes of buffer A containing 5 mM imidazole, and Survivin was eluted with 5 volumes of buffer A containing 250 mM imidazole. Samples of eluted fractions (10 µL) were loaded onto 10–20% Tris-glycine polyacrylamide gels (Invitrogen) for analysis of co-eluted proteins. Proteins were detected by Coomassie staining. Confirmation of Borealin was by Western blot, using an anti-Strep-tag primary antibody (Qiagen) followed by a rabbit anti-mouse secondary antibody conjugated to HRP (Zymed).

*Large-scale purification of complexes and crystallization.* Two complexes (full-length his$_T$-Survivin, plus strep-tagged-Borealin$^{1–120}$ and his$_T$-Survivin$^{1–120}$ plus GB1-Borealin$^{1–120}$) were expressed and purified on Ni-NTA as described above. GB1- and his$_T$-tags were removed by thrombin cleavage overnight. The proteins were further purified by size-exclusion chromatography on Sephacryl S-200 (GE Healthcare) in buffer A,
then concentrated to 4 and 17 mg/mL, respectively. Concentrated complexes were dialyzed into buffer B (100 mM NaCl, 20 mM Tris, pH 7.5, 0.1 mM TCEP). Protein identities were verified by mass spectrometry and N-terminal sequencing. Note that removal of the GB1 tag was initially incomplete but that further slow cleavage occurred upon concentration, such that there was no tag present in the crystallized complex. Full sequences for expressed proteins and final cleaved chains are shown in supplementary Scheme 1.

Crystals of Borealin20-78 in complex with Survivin1-142 were grown by vapor diffusion in sitting drops containing 0.5 µL protein and 0.5 µL well solution (0.1 M NaHEPES, pH 7.5; 0.2 M CaCl2; 28% (w/v) PEG 400). The well solution for the complex with Survivin1-120 was 0.1 M HEPES, pH 7.5; 10% (w/v) PEG 8000; 8% (v/v) ethylene glycol. Crystals were obtained after approximately 2 days at 19 °C. Prior to data collection, crystals were immersed in artificial mother liquor consisting of the well solution plus 35% PEG 400 (Survivin1-142 complex) or 20% glycerol (Survivin1-120 complex) and flash cooled in liquid nitrogen.

Data collection and structure solution. A data set for the Survivin1-142-Borealin crystals was collected at beamline 5.0.1 at the Advanced Light Source (Berkeley) and was processed with HKL software (18) (supplemental Table S1). The crystals belong to space group C2. The data are anisotropic with a functional resolution of ~2.4 Å. The structure was solved by molecular replacement using human survivin (PDB code: 1F3H; 4) as a search model. Initial electron density maps revealed clear helical density for Borealin. A model was built consisting of Borealin residues 21–76 and Survivin residues 5–141.

A 3.3 Å data set for the Survivin1-120-Borealin crystals was acquired at beamline 9-2 at the Stanford Synchronized Radiation Laboratory and was processed with HKL (18) (supplemental Table S1). The space group was I4122. The structure was solved by molecular replacement using the partially refined crystal structure of the Survivin1-142 complex and the program Phaser (CCP4, Daresbury, England), revealing three complexes in the asymmetric unit. Each complex has density for Borealin residues 20–76 (plus one additional N-terminal residue from the thrombin-cleavage site) and Survivin residues 5–119.

Both structures were refined with the program REFMAC5 and included TLS refinement (19). The final models have good stereochemistry: Ramachandran plots show that for the Survivin1-142 structure, 98.8% of all residues appear in the most favored or additionally allowed regions, with only 2 residues (1.2%) in generously allowed or disallowed regions (20). Likewise, for the Survivin1-120 complex, 98.2% of all residues appear in the most favored or additionally allowed regions, with only 1.7% of residues in generously allowed or disallowed regions. Coordinates for both structures have been deposited at the RCSB Protein Data Bank.3

Mitotic localization of Borealin mutants. Wild-type full-length Borealin and the Y54A; W70A mutant were each subcloned by PCR into pDONOR201 (Invitrogen) and fully sequenced, before transfer into pDEST 12 (Invitrogen) additionally modified with an N-terminal HA tag. HeLa cells (ATCC; CCL-2) were grown in DMEM high-glucose medium on 18 mm cover slips in 12-well plates for 20 h. Cells were then transfected with plasmid (0.5 µg) using FuGENE 6 (Roche) for 6 h. The medium was replaced with medium containing 2 mM thymidine, and the cells were incubated for an additional 16 h. Cells were then released from the thymidine block by replacement of the medium. After an additional 8 h, cells were fixed for 20 min in paraformaldehyde.

Fixed cells were washed with PBS, permeabilized with 0.1 % Triton X-100 for 5 min, and then blocked overnight in normal donkey serum (Jackson ImmunoResearch). Primary antibodies were rat anti-HA clone 3F10 (Roche, 1:1000), mouse anti-Aurora B (BD Biosciences, 611082; 1:200) and mouse anti-Survivin (Santa Cruz Biotechnology, sc-17779; 1:200). These were diluted in donkey serum and added to blocked coverslips for 1 h. After washing, the appropriate secondary antibody (donkey anti-rat CY2 or donkey anti-mouse CY3 at 1:600; Jackson ImmunoResearch) was added for 45 min in the presence of Hoechst 33342 (Invitrogen; 1:30000). Cells were washed and coverslips mounted on slides into ProLong Gold antifade reagent (Invitrogen). Images were captured using an Axioplan 2 microscope (Zeiss) with a 63X objective.
immersion objective and the acquisition program Slidebook (Intelligent Imaging Innovations).

RESULTS

Analysis of the 280-amino acid Borealin sequence suggests that it may be largely α-helical but does not predict any globular domains. However, it has been reported that Borealin1-141 is fully competent to bind Survivin (11). Within this N-terminal region are two potential coiled-coil sequences, one predicted strongly and a second predicted only weakly (Fig. 1a and supplemental Fig. S1). A series of truncated Borealin constructs was prepared focusing on the predicted boundaries of the helical elements. These were coexpressed with full length, his6-tagged Survivin in E.coli. Survivin was purified on Ni-NTA beads, and eluted protein was probed for copurifying Borealin fragments. A fragment as short as Borealin20-60 (encompassing the first predicted coiled coil) could be detected, but Borealin20-78 appeared to be the shortest fragment having full affinity (Fig. 1b). In addition to the strongly predicted coiled coil, this fragment includes at its C-terminus a short segment highly conserved in the single human Borealin and all other vertebrate Borealins of the more common Dasra B subtype (12) (supplemental Fig. S2). A subset of Borealin fragments was tested for binding to truncated forms of Survivin (Fig. 1b). Taken together, these results suggest that the strongest interactions occur between Borealin residues 20–78 and the region of Survivin that follows the BIR domain, including the first part of the long helix. This region also includes the Survivin homodimerization interface (Fig. 1c).

To better understand the interaction of Survivin with Borealin, we determined the X-ray crystal structures of both full-length Survivin and Survivin1-120 bound to Borealin20-78. The most striking aspect of both Survivin-Borealin complex structures is the 1:1 stoichiometry, which was unexpected based on the published structures of Survivin alone (Fig. 2a). Our complex structures show that Borealin wraps around Survivin with the N-terminal residues of Borealin (residues 21–60) forming a long helix which packs against the Survivin C-terminal helix. This long Borealin helix is followed by two additional short helices which pack against the juncture of the Survivin BIR domain and its C-terminal helix. The Borealin-binding site overlaps with the Survivin homodimerization interface (4,5,7). Thus Survivin cannot dimerize in this way when bound to Borealin. Closer examination reveals that the backbone conformation of Borealin64-73 is remarkably similar to that of Survivin92-101 in the homodimer, suggesting that this region of Borealin structurally mimics the displaced Survivin monomer (Fig. 2b).

The core of the interaction is Borealin65-74 which packs tightly against Survivin. In particular, the invariant Borealin Trp70 side chain enters an enlarged pocket on Survivin, where > 95% of its solvent-accessible surface area is buried (Fig. 2c, supplemental Fig. S3). This suggests an explanation for the observed importance of this region in the truncation experiments (Fig. 1b). A second significant contact is provided by Borealin Tyr54 (~ 70% buried) which is present near the C-terminus of the long Borealin helix. Trp70 and Tyr54 are the only Borealin residues to bury at least 100 Å² solvent-accessible surface in the complex. The total interface buried by the Survivin-Borealin interaction is large (~2800 Å² in the Survivin1-142 complex), with Survivin and Borealin contributing equally to the contact surface. In comparison, ~1000 Å² is buried in the Survivin homodimer interface (5). This suggests that the interaction of Survivin with Borealin is stronger than the interaction of Survivin with itself. Uncomplexed Borealin20-78 was insoluble (precluding equilibrium affinity measurements), but the purification of the heterocomplex from a large excess of expressed Survivin (data not shown) supports a higher affinity interaction. Interestingly, the recognition of Borealin by Survivin is very different from typical IAP-type peptide-binding pockets on other BIR domains (such as XIAP or ML-IAP) (Fig. 2d).

Although the major interaction between Borealin and Survivin appears to be the 1:1 complex described above, we see a crystal packing interaction for the Survivin1-120 complex that suggests the possibility of antiparallel 2:2 complex formation (supplemental Fig. S4). This interaction involves predominantly the long helices of two Borealin subunits which form extensive interhelical packing interactions. Notably, as discussed above, it does not involve
homodimerization through the interface present in published Survivin structures. (Indeed, there are no Survivin-Survivin contacts in this crystal packing.) A model of a dimer of the Survivin1-142 complex based on the 2:2 packing interaction suggests that it might be possible for the full-length complex to dimerize in this way, but that it would require changes in the degree of kinking or supercoiling of the long helices (supplemental Fig. S4C). We see a second possible antiparallel 2:2 arrangement in the crystal packing of the Survivin1-142 complex. Somewhat surprisingly, this contact does not involve any intimate interhelical packing between the 1:1 complexes, but instead involves BIR-BIR and BIR-helix contacts between Survivin monomers (supplemental Fig. S4D). However, once again, these Survivin-Survivin contacts do not involve any of the previously reported Survivin dimeric crystal contacts (4–6).

We assessed the solution stoichiometry of the Survivin1-142 complex by equilibrium and velocity ultracentrifugation (supplementary Fig. S5). The data are most consistent with a mixed population of 1:1 complex and a smaller amount of aggregate of higher order than 2:2. We see no significant population of 2:2 complex but cannot rule out the possibility that it might form under some conditions. Instead, the observed tendency of the binary complex to aggregate suggested the presence of an additional binding surface and the possibility that our Borealin fragment might be sufficient to recruit INCENP to Survivin. To test this, we coexpressed a previously described minimized INCENP construct GST–INCENP1-58 (14) and the binary complex. We find that Borealin 20–78 and Survivin copurify with the INCENP fragment at apparently stoichiometric levels (supplemental Fig. S6).

To test the biological significance of the major binding interface seen in our crystal structures, we introduced two mutations into Borealin that might be expected to alter its interaction with Survivin (Y54A; W70A, see Fig 2C). Given that Borealin1-60 (corresponding to the long helix) can bind weakly to full-length Survivin (Fig. 1), we tested whether the Y54A; W70A mutations affected binary complex formation between Survivin and Borealin1-20-78. Interestingly, we found that this binary complex could be purified from bacterial cultures in amounts comparable to those obtained with the wild-type fragment (not shown) indicating that the mutant fragment retained significant affinity for Survivin.

Tagged wild-type and mutant full-length Borealins were transiently transfected into HeLa cells and were determined to express equally (supplemental Fig. S7A). Immunofluorescence visualization of passenger proteins revealed that in the presence of the mutant Borealin, severe mislocalization occurred during later stages of mitosis (Fig. 3 and supplemental Fig. S7). In particular, the Borealin mutant was itself incorrectly localized, while HA-tagged wild-type Borealin appeared to localize normally with some background diffuse staining most likely caused by modest overexpression relative to the endogenous protein, see supplemental Fig. S7). In the presence of wild-type transfected Borealin, Aurora B and Survivin localized in the typical manner of the passenger proteins. In contrast, in cells transfected with the mutant Borealin, Aurora B was partly associated with chromosomes during anaphase and telophase, while Survivin was always entirely mislocalized to the chromosomes during these phases (Fig. 3).

**DISCUSSION**

Purified Survivin forms a dimer in solution (7) and crystallizes as a dimer (4,5). Mouse Survivin forms three distinct dimeric crystal packing contacts (6), one of which involves the same dimeric interface seen in the structures of human Survivin. There was much attention paid to the question of which dimer was the “correct dimer”, but at the time, mutagenesis studies failed to resolve the issue (21). In addition to our results, recent mutagenesis studies probing the role of a Survivin nuclear export signal (NES) indirectly suggest that the accepted Survivin dimer may not be present in the functioning CPC (22). This NES spans the Survivin dimer interface, and the mutants studied would be expected not only to affect function of the NES (as intended by the authors) but could also be expected to significantly destabilize the homodimer interface (especially T97E). Nevertheless, the Survivin mutants showed only minor differences in mitotic localization compared to wild type (22). Despite this, it has generally been assumed that the relevant form of Survivin in vivo is a dimer. The observation that differently tagged Survivin
variants coimmunoprecipitate lends some support to this idea (for example, 14). However, it has not yet been established what the overall stoichiometry is of the intact CPC (e.g., it could contain more than one "monomeric" Survivin). In addition, we have observed that even highly purified Survivin has an extreme tendency to form large aggregates, especially in lower ionic-strength buffers. We suggest that any report of Survivin coimmunoprecipitating must therefore be viewed with caution.

It would appear that our Borealin mutant Y54A; W70A has the capacity to act in a dominant negative manner and, therefore, that it interacts with at least one of the other passenger proteins or with a structural receptor for the complex. Our bacterial coexpression assay (with the mutant 20–78 fragment) suggests Survivin as a strong candidate. We observe prominent defects in translocation of CPC components to the central spindle and midbody in the presence of mutant Borealin. This is consistent with the recent report that binding of the C-terminus of Survivin to Borealin is especially important for targeting to these subcellular structures (17). Intriguingly, the functional defects we observe suggest that some aspect of CPC structure other than Survivin binding per se is perturbed in the presence of the mutant. Possibilities include loss of affinity for INCENP, failure of the CPC to bind to other cellular components, or a conformational change in the CPC involving the interaction surface we have mutated. We conclude that the interaction mode we have identified in our crystal structures is present in the chromosomal passenger complex in human cells and that it is important for the full function of the complex.

REFERENCES

1. Ambrosini, G., Adida, C., and Altieri, D. C. (1997) Nature Med. 3, 917–921.
2. Altieri, D. C. (2006) Curr. Opin. Cell Biol. 18, 609–615.
3. Lens, S. M. A., Vader, G., and Medema, R. H. (2006) Curr. Opin. Cell Biol. 18, 616–622.
4. Verdecia, M. A., Huang, H., Dutil, E., Kaiser, D. A., Hunter, T., and Noel, J. P. (2000) Nature Struct. Biol. 7, 602–608.
5. Chantalat, L., Skoufias, D. A., Kleman, J.-P., Jung, B., Dideberg, O., and Margolis, R. L. (2000) Mol. Cell 6, 183–189.
6. Muchmore, S. W., Chen, J., Jakob, C., Zakula, D., Matayoshi, E. D., Wu, W., Zhang, H., Li, F., Ng, S.-C., and Altieri, D. C. (2000) Mol. Cell 6, 173–182.
7. Sun, C., Nettesheim, D., Liu, Z., and Olejniczak, E. T. (2005) Biochemistry 44, 11–17.
8. Vader, G., Medema, R. H., and Lens, S. M. A. (2006) J. Cell Biol. 173, 833–837.
9. Ruchaud, S., Carmena, M., and Earnshaw, W. C. (2007) Nature Rev. Mol. Cell Biol. Published online Sept. 12, 2007; doi:10.1038/nrm2257.
10. Romano, A., Guse, A., Krascenicova, I., Schnabel, H., Schnabel, R., and Glotzer, M. (2003) J. Cell. Biol. 161, 229–236.
11. Gassman, R., Carvalho, A., Henzing, A. J., Ruchaud, S., Hudson, D. F., Honda, R., Nigg, E. A., Gerloff, D. L., and Earnshaw, W. C. (2004) J. Cell. Biol. 166, 179–191.
12. Sampath, S. C., Ohi, R., Leismann, O., Salic, A., Pozniakovski, A., and Funabiki, H. (2004) Cell 118, 187–202.

13. Faragher, A. J., Sun, X.-M., Butterworth, M., Harper, N., Mulherin, M., Ruchaud, S., Earnshaw, W. C., and Cohen, G. M. (2007) Mol. Biol. Cell 18, 1337–1347.

14. Klein, U. R., Nigg, E. A., and Gruneberg, U. (2006) Mol. Biol. Cell 17, 2547–2558.

15. Sessa, F., Mapelli, M., Ciferi, C., Tarricone, C., Areces, L. B., Schneider, T. R., Stukenberg, P. T., and Musacchio, A. (2005) Mol. Cell 18, 379–391.

16. Vader, G., Kauw, J. J. W., Medema, R. H., and Lens, S. M. A. (2006) EMBO Rep. 7, 85–92.

17. Lens, S. M. A., Rodriguez, J. A., Vader, G., Span, S. W., Giaccone, G., and Medema, R. H. (2006) Mol. Biol. Cell 17, 1897–1909.

18. Otwinowski, Z. and Minor, W. (1997) Methods Enzymol. 276, 307–326.

19. Winn, M. D., Murshudov, G. N., and Papiz, M. Z. (2003) Methods Enzymol. 374, 300–321.

20. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J Appl Crystallogr. 26, 283–291.

21. Shi, Y. (2000) Nature Struct. Biol. 7, 620–623.

22. Colnaghi, R., Connell, C. M., Barrett, R. M. A., and Wheatley, S. P. (2006) J. Biol. Chem. 281, 33450–33456.

23. Franklin, M. C.; Kadkhodayan, S.; Ackerly, H.; Alexandru, D.; Distefano, M. D.; Elliott, L. O.; Flygare, J. A.; Mausisa, G.; Okawa, D. C.; Ong, D.; Vucic, D.; Deshayes, K., and Fairbrother, W. J. (2003) Biochemistry 42, 8223–8231.

24. Delano, W. L. (2002) The PyMOL molecular graphics system DeLano Scientific, Palo Alto, CA.

FOOTNOTES

* We thank Lionel Rouge, Steven Shia, Deanne Compaan, and Mark Ultsch for assistance with crystallization and data collection. Miro Brajenovic, Rami Hannoush, Borlan Pan, and Sandrine Ruchaud provided advice on many experimental aspects. Finally, we thank Laszlo Komuves for invaluable assistance with microscopy.

1The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1-S7, supplemental Table 1, and supplemental Scheme 1.

2The abbreviations used are: BIR, baculoviral inhibitory repeat; IAP, inhibitor-of-apoptosis; CPC, chromosomal passenger complex; INCENP, inner centromere protein; SAC, spindle assembly checkpoint; IPTG, isopropyl-β-D-1-thiogalactopyranoside; TCEP, Tris-(2-carboxyethyl)phosphine; NTA, Nitrilotriacetic acid; HRP, horseradish peroxidase; HEPES, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid); PEG, polyethylene glycol; PCR, polymerase chain reaction; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; r.m.s.d, root mean-squared deviation.
The coordinates for the structure of Survivin\textsubscript{1–142} bound to Borealin\textsubscript{20–78} have been deposited with the Research Collaboratory for Structural Bioinformatics Protein Databank = PDB # 2RAW. The coordinates for the structure of Survivin\textsubscript{1–120} bound to Borealin\textsubscript{20–78} have been deposited with the Research Collaboratory for Structural Bioinformatics Protein Databank = PDB # 2RAX.

**FIGURE LEGENDS**

**Fig. 1.** Defining the minimal fragment of Borealin necessary to bind Survivin and the Survivin interaction surface. (A) Aligned primary sequences of the Borealin N-terminus. The yellow bars indicate sequences either strongly predicted (thicker bar) or weakly predicted (thinner bar) to form a coiled coil structure. Details are shown in supplemental Fig. S1. Boundary residues for the truncation constructs are indicated above the sequences. (B) Summary of fragment copurification studies. Interactions were scored based on whether Borealin could be detected readily by Coomassie staining “+”, only by Western blot “(+)”, or by neither method “−” (see Experimental Procedures). (C) Inferred interaction surface (teal) of Borealin fragment 20–78 mapped onto the previously reported dimeric Survivin structure (PDB code:1F3H; 4). The putative interaction surface spans the homodimerization interface.

**Fig. 2.** Structure of the Survivin-Borealin complex. (A) Comparison of the overall architecture of the Survivin dimer and the 1:1 Survivin-Borealin complex. At left is a different view of the Survivin homodimer than that shown in Fig. 1C, with monomers shown in purple and blue (PDB code:1F3H; 6). The 2.4 Å structure of Survivin\textsubscript{1–142} and the Borealin fragment is shown at center, while the 3.3 Å Survivin\textsubscript{1–120} is shown at right. Survivin is shown in white, and the Borealin fragment is shown in teal. (B) Close-up view of the Survivin dimer interface (far left) and the corresponding region of the Survivin\textsubscript{1–142} complex (second from left). Subunits are colored as in part A. Note the rearrangement of Survivin side chains to accommodate the Borealin W70 side chain. The overlay at second from right illustrates the backbone mimicry by Borealin of the displaced Survivin monomer, while the illustration at far right shows the Survivin-Survivin backbone overlay in greater detail. Sequences of the two segments shown at right are A\textsubscript{64}LREMNWLDY\textsubscript{73} (Borealin) and Q\textsubscript{92}FEELTLGEF\textsubscript{101} (Survivin). Colors for the Survivin homodimer and the complex subunits are as shown in the left two illustrations. (C) Surface representation of Survivin showing the pocket that opens up to accommodate Borealin W70 side chain. The solvent accessible surface of all Survivin residues with 4.2 Å of the W70 side chain is colored blue. The side chains of these Survivin residues are shown as red sticks. (D) Comparison of the mode of Borealin recognition to the typical BIR-domain peptide-binding site seen in inhibitor-of-apoptosis (IAP) proteins. The BIR domain of Survivin and the ML-IAP BIR domain (PDB code: 1OXX; 23) are superimposed (r.m.s.d. = 0.9 Å for structurally conserved BIR-domain heavy atoms). The peptide ligand bound to ML-IAP is shown in yellow sticks. There is no overlap of IAP-like peptide and Borealin binding sites on the surface of the Survivin monomer. All structure figures were produced using the program PyMOL (24).

**Fig. 3.** Mutagenesis of key Borealin residues buried in the interface with Survivin and assessment of chromosomal passenger localization during anaphase and telophase. Wild-type HA-tagged Borealin or the Y54A; W70A mutant was transfected into HeLa cells as described in Experimental Procedures. Cells were stained with anti-HA antibody, antibodies to Aurora B or Survivin, and Hoechst 33342 to visualize chromosomes. The images shown are for the endogenous passenger protein (Aurora B or Survivin) and for the overlay of passenger protein with DNA and HA-staining. A complete set of images is given in supplemental Fig. S7. (A) Comparison of mutant and wild-type Borealin effects on Aurora B and Survivin localization during anaphase. In the presence of wild-type HA-Borealin, both Aurora B and Survivin localize in punctate regions at the central spindle. When cells are transfected instead with mutant HA-Borealin, both Aurora B and Survivin appear instead to associate predominantly with chromosomes. A minor fraction of Aurora B can be seen in its normal location at the central spindle region, but Survivin appears completely mislocalized. (B) Comparison of mutant and wild-type Borealin effects on Aurora B
and Survivin localization during telophase. In the presence of wild-type HA-Borealin, Aurora B and Survivin are able to translocate to the midbody and are seen in two bright spots flanking the central point. In the presence of the HA-Borealin mutant, the localization of Survivin is very strongly affected, with Survivin appearing to be completely absent from the midbody. Again, Survivin appears instead to localize with chromosomal DNA. Aurora B localization is partially affected. Like Survivin, it appears to be significantly associated with the chromosomes. However, it can be seen also in the midbody (with much less intense staining than normal).
Figure 1
Figure 2
Figure 3
The mitotic regulator survivin binds as a monomer to its functional interactor Borealin
Eric Bourhis, Sarah G. Hymowitz and Andrea G. Cochran

*J. Biol. Chem.* published online September 19, 2007

Access the most updated version of this article at doi: 10.1074/jbc.M706233200

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

Supplemental material:
http://www.jbc.org/content/suppl/2007/09/21/M706233200.DC1