The emergence of protein-tyrosine phosphatase 1B (PTP1B) as a potential drug target for treatment of diabetes, obesity, and cancer underlies the importance of understanding its full range of cellular functions. Here, we have identified cortactin, a central regulator of actin cytoskeletal dynamics, as a substrate of PTP1B. A trapping mutant of PTP1B binds cortactin at the phosphorylation site Tyr446, the regulation and function of which have not previously been characterized. We show that phosphorylation of cortactin Tyr446 is induced by hyperosmolarity and potentiates apoptotic signaling during prolonged hyperosmotic stress. This study advances the importance of Tyr446 in the regulation of cortactin and provides a potential mechanism to explain the effects of PTP1B on processes including cell adhesion, migration, and tumorigenesis.

Protein-tyrosine phosphatase (PTP)4-1B is recognized as an important regulator of metabolic signaling in mice. Ablation of the gene encoding PTP1B, Ptpn1, causes tissue-specific hypersensitivity to insulin and leptin, resulting in resistance to diabetes and obesity (1, 2). PTP1B is a ubiquitously expressed enzyme that is localized to the cytoplasmic face of the endoplasmic reticulum (3). Its catalytic domain can directly dephosphorylate and inactivate the insulin receptor and other receptor and non-receptor protein-tyrosine kinases (4). Despite the proto-oncogenic functions of many of these protein-tyrosine kinases, Ptpn1-null mice are not prone to tumorigenesis. On the contrary, two recent studies found that mice lacking PTP1B are markedly resistant to mammary tumorigenesis induced by active mutants of the receptor tyrosine kinase ErbB2 (5, 6).

The effect of PTP1B deficiency in these cancer models has highlighted its diverse cellular functions. One emerging role of PTP1B is in the regulation of the actin cytoskeleton. Early studies in fibroblasts showed that PTP1B is required for proper cell adhesion and spreading on extracellular matrix proteins (7). Cell adhesion to the extracellular matrix is mediated largely by cell-surface integrins, which initiate signaling cascades and orchestrate changes in the actin cytoskeleton at adhesive contacts. Protein-tyrosine kinases involved in transmission of integrin signals, including c-Src (8), focal adhesion kinase (9), and Csk (10), have been proposed as PTP1B substrates; however, none has consistently been shown to be hyperphosphorylated in the absence of PTP1B. It is tempting to speculate that, rather than regulating upstream signaling, PTP1B may directly target one or more non-protein-tyrosine kinase actin regulatory proteins.

Cortactin was identified as a prominent tyrosine phosphoprotein in cells expressing the active protein-tyrosine kinase v-Src (11). It has subsequently been implicated in various processes requiring dynamic actin assembly, including cell adhesion and migration, vesicular transport, and microbial infection (12, 13). Cortactin exerts its effects on the actin cytoskeleton by interacting directly with the Arp2/3 complex (via its N-terminal acidic domain) (see Fig. 3), F-actin (via a central repeat region), and other actin regulatory proteins such as N-WASP and MIM (via its C-terminal SH3 domain) (12). A variety of additional binding partners mediate the effects of cortactin in specific contexts, including receptor tyrosine kinase down-regulation and cell-cell adhesion.

Cortactin is tyrosine-phosphorylated in response to a wide range of stimuli that induce cytoskeletal rearrangement, including growth factor stimulation, cell adhesion, and hyperosmotic stress (14). Src phosphorylates murine cortactin predominantly at three key sites in vitro, Tyr421, Tyr466, and Tyr482 (corresponding to Tyr421, Tyr470, and Tyr486 in human cortactin), resulting in decreased actin cross-linking activity (15). The combined mutation of these three residues abolishes tyrosine phosphorylation of cortactin in cells under various conditions (15–17). Thus, these Src sites have been the focus for functional characterization of cortactin tyrosine phosphorylation. Nonetheless, several mass spectrometry-phosphoproteomic studies have identified additional phosphorylated tyrosine residues (18–24). A number of individual phosphotyrosine sites have been reported independently in different cell types and in response to diverse stimuli, but their regulation and function remain to be investigated.

In this study, we have identified cortactin as a substrate of PTP1B. A trapping mutant of PTP1B binds cortactin at a previously uncharacterized tyrosine residue, Tyr446. We show that PTP1B regulates cortactin phosphorylation induced by hyperosmolarity and that Tyr446 is required for protection from apo-
ptosis induced by hyperosmotic stress. Our results are the first to implicate a specific PTP in the regulation of cortactin, and they reveal a novel mechanism by which PTP1B may influence the cytoskeleton.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—The following antibodies were used: anti-cortactin clone 4F11 and anti-phosphotyrosine clone 4G10 (Millipore), anti-cortactin phospho-Tyr<sup>212</sup> (Invitrogen), anti-PTP1B clone 15 (BD Transduction Laboratories), and anti-GST (Z-5, Santa Cruz Biotechnology, Inc.). An anti-cortactin polyclonal antibody (used exclusively for supplemental Fig. 1) was purchased from Cell Signaling Technology. The polyclonal antibody specific for human cortactin phospho-Tyr<sup>446</sup> was prepared by Quality Controlled Biochemicals (Hopkinton, MA).

**Plasmids**—Expression plasmids (pEBG) encoding GST-tagged human PTP1B (WT and D181A) were described previously (25). To prepare additional GST-tagged PTP constructs, cDNAs from pEF-BOS-TCPTP (WT and D182A) and pcDNA4-PTP-PEST (WT and D199A) were amplified by PCR, introducing BamHI (5′) and NotI (3′) sites, and subsequently subcloned into the pEBG vector. Untagged expression constructs (pcDNA3) encoding human WT cortactin and mutants Y421F, Y470F, and Y486F were a kind gift of Dr. Scott Weed (West Virginia University). Additional cortactin mutants were prepared by site-directed mutagenesis (QuikChange kit, Stratagene). Plasmids used for expression of untagged human PTP1B (WT or C215S, pcDNA3.1) were described previously (26). Insert sequences of all constructs were confirmed by DNA sequencing.<sup>5</sup>

**Substrate Trapping**—Cell lines were routinely maintained in DMEM containing 10% fetal bovine serum and 50 μg/ml gentamicin (all from Invitrogen). For trapping of endogenous cortactin, HeLa or COS-7 cells (plated at 5 × 10<sup>5</sup> cells/6-cm dish) were transfected with 4.8 μg of DNA (4 μg of pEBG-PTP1B and 0.8 μg of v-Src or a control empty vector) using Lipofectamine 2000 (Invitrogen) following the manufacturer’s protocol. For GST-PTP/cortactin coexpression experiments, COS-7 cells (2.5 × 10<sup>5</sup>/well in 6-well plates) were transfected with 1 μg of pEBG-PTP and 1 μg of pcDNA3-cortactin DNA. GST pull-down assays were performed as described (27).

**EGF and Sucrose Stimulation**—For overexpression experiments, HeLa cells (total of 10<sup>6</sup> cells/12-well plate) were transfected with 0.5 μg of pcDNA3-cortactin (WT or Y446F), pcDNA3.1-PTP1B (WT or C215S), or a control empty vector (pcDNA3.1) and grown for 36 h in normal growth medium. Cells were then serum-starved in DMEM for 4 h prior to treatment with hyperosmotic sucrose (300 mM sucrose in DMEM) or human EGF (100 nM; R&D Systems). For inhibitor experiments, untransfected HeLa cells were serum-starved for 2 h in DMEM followed by 2 h in DMEM containing a cell-permeable PTP1B inhibitor (5 μM final concentration) as described previously (28). The hyperosmotic sucrose medium used for cell stimulation contained the same concentration of inhibitor. For all experiments, cells were lysed, and protein extracts were analyzed by immunoblotting as described previously (26).

**Apoptosis Assays**—HeLa cells plated in 6-cm dishes were transfected with pcDNA3-cortactin (WT or Y446F). At 24 h post-transfection, cells were trypsinized and seeded at 10<sup>4</sup> cells/well in a 96-well plate. Cells were allowed to adhere for 10 h and subsequently serum-starved in 0.1% fetal bovine serum-containing DMEM for 16 h prior to treatment with hyperosmotic sucrose (300 mM in DMEM). At the indicated time points, caspase activity was measured using the luminescent Caspase-Glo 3/7 assay (Promega) following the manufacturer’s protocol.

**RESULTS**

**Identification of Cortactin as a PTP1B Substrate**—To identify novel PTP1B substrates, a cell-based pulldown assay was employed in which GST-tagged WT PTP1B or mutant D181A was coexpressed in COS-7 cells with v-Src. The interaction of WT PTP1B with its substrates is normally transient. Asp<sup>181</sup> is a critical catalytic residue, and the D181A trapping mutant is able to form a stable, covalently linked complex with phosphorylated target proteins. The proteins bound to these two forms of PTP1B were purified using glutathione beads. As expected, the total cell lysate of COS-7 cells expressing v-Src contained numerous tyrosine phosphoproteins, as demonstrated by immunoblotting with an anti-phosphotyrosine antibody (Fig. 1A, *third lane*). Although WT PTP1B did not associate significantly with any of these proteins (*first lane*), the D181A mutant bound preferentially to two phosphoproteins of ~80 and ~65 kDa (*second lane*).

By immunoblotting for candidate substrates, we found that cortactin bound PTP1B D181A and that its molecular mass precisely matches the ~80-kDa protein we had detected by anti-phosphotyrosine blotting. The trapping mutant of PTP1B was able to bind endogenous cortactin in both HeLa and COS-7 cells (Fig. 1B). The form of cortactin expressed in COS-7 (monocytic) cells migrated somewhat slower than that in HeLa (human) cells. An alternative antibody that selectively detects human cortactin was used to confirm binding of PTP1B D181A to cortactin in HeLa cells (supplemental Fig. 1). In both cell types, expression of v-Src enhanced, but was not required, for the cortactin-PTP1B interaction, suggesting that the basal phosphorylation of cortactin is sufficient for binding. An association of WT PTP1B with cortactin was never detected, even after long exposures, indicating that like many of its substrates, cortactin and WT PTP1B do not form a stable complex.

PTP1B belongs to the family of 38 classical, phosphotyrosine-specific PTPs (29). We examined the specificity of the cortactin-PTP1B interaction by testing the binding of cortactin to two other classical PTPs, TCPTP and PTP-PEST. TCPTP is the most similar enzyme to PTP1B, and although it is predominantly nuclearly localized, many of its known substrates, including receptor tyrosine kinases and STAT (signal transducer and activator of transcription) transcription factors, overlap with PTP1B (4). PTP-PEST is a cytoplasmic PTP that is a well established regulator of the actin cytoskeleton. The WT and trapping mutant forms of PTP1B, TCPTP, and PTP-PEST were coexpressed as GST fusion proteins in COS-7 cells with WT cortactin (Fig. 2). After precipitation using glutathione

---

<sup>5</sup>The sequences of primers used for cloning and mutagenesis are available on request.
beads, bound cortactin was detected only in cells expressing PTP1B D181A. Thus, among the candidate enzymes tested, the ability to interact with cortactin is specific to PTP1B.

**Mapping of the Cortactin-PTP1B Interaction**—To localize the PTP1B-binding site on cortactin, we tested whether cortactin deletion mutants lacking the SH3 domain or the entire C terminus (Fig. 3A) could be trapped by PTP1B D181A. Despite potential interactions between the cortactin SH3 domain and the PTP1B proline-rich motifs, the ΔSH3 mutant retained binding to PTP1B (Fig. 3B). Similarly, deletion of either proline-rich motif of PTP1B D181A did not impair its ability to pull down cortactin (data not shown). In contrast, deletion of the cortactin C terminus, including the SH3 and α-helical/proline-rich regions, prevented the binding of PTP1B (Fig. 3B). The α-helical/proline-rich region contains a cluster of tyrosine residues including the canonical Src sites (Fig. 3A). Tyrosine-to-phenylalanine mutants were generated for each of these residues, and binding to PTP1B D181A was assessed after cotransfection. Strikingly, mutation of a single tyrosine residue, Tyr446, nearly abolished the interaction (Fig. 3C). The results of independent binding assays were quantified by densitometry (Fig. 3D). Interestingly, mutants at two of the Src sites, Y421F and Y486F, displayed comparable binding to PTP1B D181A, whereas another Src site mutant, Y470F, as well as Y464F and Y489F, had intermediate effects on the interaction. These data strongly suggest that Tyr446 is the primary binding site, whereas additional residues may indirectly affect binding, perhaps by altering the phosphorylation state of Tyr446.

**Development of a Phospho-specific Antibody for Cortactin Tyr446**—Phosphoproteomic studies have identified cortactin peptides phosphorylated at Tyr446 (18–22, 24), and multiple species alignment of cortactin protein sequences indicates that Tyr446 has been evolutionarily conserved (Fig. 4A). These results suggest that Tyr446 is a functionally important site for...
the regulation of cortactin. To evaluate the role of PTP1B in modulating cortactin phosphorylation, a polyclonal antibody for human cortactin phospho-Tyr446 was prepared by immunization of rabbits with a corresponding phosphopeptide (Fig. 4A). The specificity of the anti-phospho-Tyr446 antibody was tested using lysates from transfected HeLa cells treated with EGF. The overexpressed cortactin, which corresponds to the long isoform of the human protein (30), migrated slightly slower on SDS-polyacrylamide gel than the endogenous form expressed in these cells. Immunoblot analysis showed that the antibody bound endogenous and overexpressed WT cortactin, but not the overexpressed Y446F mutant (Fig. 4B). The reactivity of the antibody with cortactin was increased with EGF treatment, reaching a maximum at a stimulation time of 15 min. These results confirm that Tyr446 is a target of EGF signaling and that this novel phospho-specific antibody is specific for this site.

PTP1B Regulates Cortactin Tyrosine Phosphorylation—In initial experiments, we found that PTP1B overexpression dramatically decreased cortactin phosphorylation in response to EGF (data not shown). However, the EGF receptor and other receptor tyrosine kinases that promote cortactin phosphorylation are themselves substrates of PTP1B. Thus, the effect of PTP1B overexpression in this context is likely due to its cumulative activity on upstream receptor tyrosine kinases as well as on cortactin itself.

Hyperosmotic media can potently induce tyrosine phosphorylation of cortactin independently of receptor tyrosine kinases. Cell shrinkage caused by hyperosmolality leads to successive activation of the Fyn and Fer cytoplasmic protein-tyrosine kinases (16). Activated Fer was believed to phosphorylate cortactin at the previously identified Src sites, Tyr421, Tyr470, and Tyr486 (16). However, our results show that Tyr446 is also targeted under these conditions: treatment with hyperosmotic sucrose induced Tyr446 phosphorylation in HeLa cells expressing WT cortactin (endogenous or overexpressed) (Fig. 5A). Immunoblotting with an antibody specific for Tyr421 showed that, as expected, this site was also phosphorylated in response to hyperosmolality. Notably, we found that the Y446F mutation resulted in decreased Tyr421 phosphorylation (Fig. 5A), suggesting that the phosphorylation state of these residues is interdependent.

To establish the role of PTP1B in this process, we tested whether altering its expression or activity affects hyperosmolality-induced cortactin phosphorylation. Transient overexpression of WT PTP1B reduced phosphorylation at both Tyr446 and Tyr471 (Fig. 5B), whereas an inactive mutant of PTP1B (C215S) had no effect. Correspondingly, preincubation of cells with a membrane-permeable PTP1B inhibitor enhanced hyperosmolality-induced cortactin phosphorylation (16). However, our results show that Tyr446 is also targeted under these conditions: treatment with hyperosmotic sucrose induced Tyr446 phosphorylation in HeLa cells expressing WT cortactin (endogenous or overexpressed) (Fig. 5A). Immunoblotting with an antibody specific for Tyr421 showed that, as expected, this site was also phosphorylated in response to hyperosmolality. Notably, we found that the Y446F mutation resulted in decreased Tyr421 phosphorylation (Fig. 5A), suggesting that the phosphorylation state of these residues is interdependent.

To establish the role of PTP1B in this process, we tested whether altering its expression or activity affects hyperosmolality-induced cortactin phosphorylation. Transient overexpression of WT PTP1B reduced phosphorylation at both Tyr446 and Tyr471 (Fig. 5B), whereas an inactive mutant of PTP1B (C215S) had no effect. Correspondingly, preincubation of cells with a membrane-permeable PTP1B inhibitor enhanced hyperosmolality-induced cortactin phosphorylation (16). However, our results show that Tyr446 is also targeted under these conditions: treatment with hyperosmotic sucrose induced Tyr446 phosphorylation in HeLa cells expressing WT cortactin (endogenous or overexpressed) (Fig. 5A). Immunoblotting with an antibody specific for Tyr421 showed that, as expected, this site was also phosphorylated in response to hyperosmolality. Notably, we found that the Y446F mutation resulted in decreased Tyr421 phosphorylation (Fig. 5A), suggesting that the phosphorylation state of these residues is interdependent.

FIGURE 3. PTP1B D181A binds cortactin at Tyr446. A, the structure of full-length (FL) human cortactin is shown, with the positions of the 6.5 tandem repeats (TR), the α-helical/proline-rich (AHPR) region, and the C-terminal (C-term) SH3 domain indicated. The α-helical/proline-rich region contains nine tyrosine residues, including the three Src sites (denoted by asterisks). B, deletion of the entire C terminus of cortactin, but not the SH3 domain alone, disrupts binding to PTP1B. WT cortactin and mutants were coexpressed in COS-7 cells with GST-PTP1B (D181A or WT), and interacting proteins were detected by immunoblotting (IB) after GST pulldown (PD). C, mutation of cortactin at Tyr446 blocks binding of PTP1B. GST-PTP1B D181A-cortactin binding was tested as described for B. For the control (Cont) assay, GST-WT PTP1B was coexpressed with WT cortactin. D, the results of three independent binding experiments are quantified. The band density of cortactin isolated by pulldown and in the total cell lysate (TCL) was quantified by densitometry. Error bars represent S.E.
Mutation of Cortactin Tyr^{446} Increases Hyperosmolarity-induced Apoptosis—Remodeling of the actin cytoskeleton is a protective mechanism in cells exposed to a hyperosmotic environment. Nonetheless, prolonged exposure leads to apoptotic cell death in a variety of cell types (31). A recent study showed that a single tyrosine residue on the protein-tyrosine kinase focal adhesion kinase, phosphorylated during hyperosmotic stress, is important for protection from apoptosis (32). Because cortactin is tyrosine-phosphorylated in response to hyperosmolarity (33), we investigated whether cortactin Tyr^{446} could also play a cytoprotective role. Hyperosmotic sucrose induced apoptosis in HeLa cells as indicated by robust caspase-3/7 activation (Fig. 6A). After a 6-h treatment, transient overexpression of cortactin Y446F resulted in a significant ~30% increase in caspase activation compared with cells overexpressing the WT protein (Fig. 6B). This result indicates that mutation of Tyr^{446} potentiates apoptotic signaling in HeLa cells under hyperosmotic stress.

DISCUSSION

The effect of PTP1B on the onset of diabetes, obesity, and breast tumorigenesis in mice has focused attention on its potential as a therapeutic target. However, the mechanisms by which it accomplishes some of its known cellular functions, including the modulation of cell-extracellular matrix adhesion, remain unclear. Here, we have shown that the actin regulatory protein cortactin is a target of PTP1B. In COS-7 cells expressing v-Src, the D181A trapping mutant of PTP1B binds selectively to cortactin, at ~80 kDa, and a second protein of ~65 kDa. This degree of specificity is remarkable considering the multitude of tyrosine phosphoproteins present in these cells and the numerous known substrates of PTP1B. Previously, our laboratory identified p62Dok as a PTP1B substrate in mouse fibroblasts using a similar approach (34). However, on the basis of immunoblotting data (not shown), we suspect that the ~65-kDa protein is not p62Dok, and we are currently investigating its identity.

The ability of PTP1B to bind cortactin is not shared by the related enzymes TCPTP and PTP-PEST. The specificity of the cortactin-PTP1B interaction is further demonstrated by the fact that their binding depends largely on a single tyrosine residue, Tyr^{446}. Published phosphoproteomic data provide convincing evidence that cortactin Tyr^{446} is a bona fide phosphorylation site: phospho-Tyr^{446} peptides have been detected following activation or overexpression of receptor tyrosine kinases (18, 20, 21, 23) as well as in cells treated with pervanadate, a general PTP inhibitor (19, 22, 24). Here, using immunological

FIGURE 4. Development of a phospho-antibody specific for Tyr^{446} of human cortactin. A, Tyr^{446} of human cortactin is highly conserved between species. A polyclonal antibody was produced by immunizing rabbits with a peptide corresponding to the sequence surrounding Tyr^{446} (boxed). B, Tyr^{446} is phosphorylated in response to EGF. HeLa cells overexpressing WT cortactin or Y446F were treated with EGF for the indicated times, and lysates were analyzed by immunoblotting (IB).

FIGURE 5. PTP1B regulates sucrose-induced phosphorylation of cortactin. A, cortactin (cort) Tyr^{446} is phosphorylated in response to hyperosmolarity. HeLa cells were transiently transfected with expression constructs encoding WT cortactin and Y446F or an empty vector and subsequently stimulated with 300 mM sucrose (Suc) for the indicated times. Lysates were analyzed by immunoblotting (IB) with the indicated antibodies (B). Hyperosmolarity-induced cortactin phosphorylation is reduced by WT but not inactive PTP1B. HeLa cells were transfected with expression constructs encoding WT PTP1B and C215S (CS) or an empty vector and stimulated as described for B. C, PTP1B inhibition increases hyperosmolarity-induced cortactin phosphorylation. HeLa cells were treated with a small molecule PTP1B inhibitor prior to stimulation with sucrose as described for A. D, quantitation of four independent experiments described in C. Error bars represent S.E. Asterisks denote data points that are significantly greater than the control points (*p < 0.05) calculated using an unpaired, two-tailed, Student's t test.
PTP1B-mediated Regulation of Cortactin

In conclusion, we have shown that PTP1B regulates cortactin tyrosine phosphorylation, likely by directly dephosphorylating Tyr446. This is the first report implicating a specific enzyme in the modification of this site, and it also establishes its functional significance in the protection of cells during hyperosmotic stress. On the basis of these results and published phosphoproteomic data, we propose that the current model of cortactin regulation by tyrosine phosphorylation, which holds that Tyr421, Tyr470, and Tyr486 are of primary importance, should be revised to include Tyr446. Furthermore, this study suggests a novel mechanism by which PTP1B may affect a variety of cellular processes, including tumorigenesis, through regulation of the actin cytoskeleton.

Acknowledgments—We thank Maxime Hallé for helpful discussions, Drs. Veena Sangwan and Morag Park for suggestions and for critical reading of the manuscript, and Dr. Scott Weed for providing the human cortactin expression plasmids. We are grateful to Merck Frosst (Kirkland, Quebec) for supplying the PTP1B inhibitor.

REFERENCES

1. Elchebly, M., Payette, P., Michaliszyn, E., Cromlish, W., Collins, S., Loy, A. L., Normandin, D., Cheng, A., Himms-Hagen, J., Chan, C. C., Ramachandran, C., Gresser, M. J., Tremblay, M. L., and Kennedy, B. P. (1999) Science 283, 1544–1548
2. Benec, K. K., Delibegovic, M., Xue, B., Gorgun, C. Z., Hotamisligil, G. S., Neel, B. G., and Kahn, B. B. (2006) Nat. Med. 12, 917–924
3. Frangioni, J. V., Heahm, P. H., Shifrin, V., Jost, C. A., and Neel, B. G. (1992) Cell 68, 545–560
4. Blanchetot, C., Chagnon, M., Dubé, N., Hallé, M., and Tremblay, M. L. (2005) Methods (San Diego) 35, 44–53
5. Julien, S. G., Dube´, N., Read, M., Penney, J., Paquet, M., Han, Y., Kennedy, B. P., Muller, W. J., and Tremblay, M. L. (2007) Nat. Genet. 39, 338–346
6. Bentires-Alj, M., and Neel, B. G. (2007) Cancer Res. 67, 2420–2424
7. Cheng, A., Bal, G. S., Kennedy, B. P., and Tremblay, M. L. (2001) J. Biol. Chem. 276, 25848–25855
8. Bjorge, J. D., Pang, A., and Fujita, D. J. (2000) J. Biol. Chem. 275, 41439–41446
9. Zhang, Z., Lin, S. Y., Neel, B. G., and Haimovich, B. (2006) J. Biol. Chem. 281, 1746–1754
10. Arias-Salgado, E. G., Hag, F., Dubois, C., Morain, B., Kasirer-Friede, A., Fure, B. C., Fure, B., Neel, B. G., and Shattil, S. J. (2005) J. Cell Biol. 170, 837–845
11. Wu, H., Reynolds, A. B., Kanner, S. B., Vines, R. R., and Parsons, J. T. (1991) Mol. Cell. Biol. 11, 5113–5124
12. Buday, L., and Downward, J. (2007) Biochim. Biophys. Acta 1775, 263–273
13. Selbach, M., and Backert, S. (2005) Trends Microbiol. 13, 181–189
14. Liu, B. L., and Low, B. C. (2005) FEBS Lett. 579, 577–585
15. Huang, C., Liu, J., Haudenschild, C. C., and Zhan, X. (1998) J. Biol. Chem. 273, 25770–25776
16. Kapus, A., Di Ciano, C., Sun, J., Zhan, X., Kim, L., Wong, T. W., and Rotstein, O. D. (2000) J. Biol. Chem. 275, 32289–32298
17. Fan, L., Di Ciano-Oliveira, C., Weed, S. A., Craig, A. W., Greer, P. A., Rotstein, O. D., and Kapus, A. (2004) Biochem. J. 380, 581–591

methods, we have confirmed that Tyr446 is a target of EGF receptor signaling and shown that it is also phosphorylated in response to hyperosmotic stress.

There is evidence that cortactin is phosphorylated in a progressive manner, with phospho-Tyr421 potentially acting as a docking site for Src, allowing it to phosphorylate Tyr470 (35). However, it seems unlikely that Tyr446 represents an additional secondary site. First, mass spectrometry studies have identified, in several cases, Tyr446 as the sole tyrosine-phosphorylated cortactin residue (20, 21, 24). Second, our results show that in response to hyperosmolarity, the phosphorylation state of Tyr421 is dependent on the presence of Tyr446. Correspondingly, although the trapping mutant of PTP1B binds to Tyr446 and not Tyr421, inhibition of PTP1B or overexpression of the WT enzyme affects both residues. Finally, we have shown that Tyr446 is required for protection of cells from hyperosmolarity-induced apoptosis, pointing to the potential importance of this single residue in the regulation of actin remodeling. In future studies, we plan to investigate further the interrelationship between Tyr446 and the canonical Src sites as well as whether particular effectors of cortactin function depend on Tyr446 phosphorylation.

An exciting possibility is that the regulation of cortactin could contribute to the effect of PTP1B on breast tumorigenesis in mice (5, 6). Amplification of a genomic region containing EMS1, encoding human cortactin, is associated with human cancer, and cortactin has been implicated in the promotion of tumor cell migration and invasion (12). It will be interesting to determine whether expression of cortactin Y446F affects the tumorigenicity of cancer cells. Nonetheless, deciphering the specific importance of PTP1B-mediated cortactin regulation in this process will be a challenge, given the pleiotropic effects of this enzyme on cancer cell signaling. Potentially, the phosphorylation state of Tyr446 could also be used as an in vivo reporter to monitor the effectiveness of PTP1B-targeted therapies.

FIGURE 6. Cortactin Tyr446 is required for resistance to hyperosmotic stress-induced apoptosis. A, hyperosmotic sucrose triggers apoptosis in HeLa cells. Cells were serum-starved and treated with 300 mM sucrose for the indicated times prior to detection of caspase-3/7 activity. RLU, relative light units. B, HeLa cells transiently overexpressing WT cortactin or Y446F were treated as described for A. For each experiment, caspase activity measurements were normalized to the WT cortactin 3-h time point. Values represent the average of three independent experiments, and error bars correspond to S.E. The asterisk denotes a statistically significant difference (p < 0.05) in caspase-3/7 activity between WT cortactin- and Y446F-expressing cells calculated using an unpaired, two-tailed, Student’s t test.

JUNE 6, 2008 • VOLUME 283 • NUMBER 23 JOURNAL OF BIOLOGICAL CHEMISTRY 15745
**PTP1B-mediated Regulation of Cortactin**

18. Wolf-Yadlin, A., Hautaniemi, S., Lauffenburger, D. A., and White, F. M. (2007) *Proc. Natl. Acad. Sci. U. S. A.* **104**, 5860–5865

19. Rush, J., Moritz, A., Lee, K. A., Guo, A., Goss, V. L., Spek, E. J., Zhang, H., Zha, X. M., Polakiewicz, R. D., and Comb, M. J. (2005) *Nat. Biotechnol.* **23**, 94–101

20. Hinsby, A. M., Olsen, J. V., Bennett, K. L., and Mann, M. (2003) *Mol. Cell. Proteomics** **2**, 29–36

21. Zhang, Y., Wolf-Yadlin, A., Ross, P. L., Pappin, D. J., Rush, J., Lauffenburger, D. A., and White, F. M. (2005) *Mol. Cell. Proteomics** **4**, 1240–1250

22. Amanchry, R., Kalume, D. E., Iwahori, A., Zhong, J., and Pandey, A. (2005) *J. Proteome Res.* **4**, 1661–1671

23. Wolf-Yadlin, A., Kumar, N., Zhang, Y., Hautaniemi, S., Zaman, M., Kim, H. D., Grantcharova, V., Lauffenburger, D. A., and White, F. M. (2006) *Mol. Syst. Biol.* **2**, 54

24. Wang, Y., Du, D., Fang, L., Yang, G., Zhang, C., Zeng, R., Ullrich, A., Lottspeich, F., and Chen, Z. (2006) *EMBO J.* **25**, 5058–5070

25. Simoncic, P. D., Lee-Loy, A., Barber, D. L., Tremblay, M. L., and McGlade, C. J. (2002) *Curr. Biol.* **12**, 446–455

26. Stauble, M., Zhao, L., Aubry, I., Schmidt-Arras, D., Bohn, F. D., Li, C. J., and Tremblay, M. L. (2007) *ChemBioChem* **8**, 179–186

27. Zhang, Y., Wolf-Yadlin, A., Ross, P. L., Pappin, D. J., Rush, J., Lauffenburger, D. A., and White, F. M. (2005) *Mol. Cell. Proteomics** **4**, 1240–1250

28. Montalibet, J., Skorey, K., McKay, D., Scapin, G., Asante-Appiah, E., and Kennedy, B. P. (2006) *J. Biol. Chem.* **281**, 5258–5266

29. Alonso, A., Sasin, J., Bottini, N., Friedberg, L., Osterman, A., Godzik, A., Hunter, T., Dixon, J., and Mustelin, T. (2004) *Cell* **117**, 699–711

30. van Rossum, A. G., de Graaf, J. H., Schuuring-Scholetes, E., Klun, P. M., Fan, Y. X., Zhan, X., Moojenaar, W. H., and Schuuring, E. (2003) *J. Biol. Chem.* **278**, 45672–45679

31. Burg, M. B., Ferraris, J. D., and Dmitrieva, N. I. (2007) *Physiol. Rev.* **87**, 1441–1474

32. Lunn, J. A., Jacamo, R., and Rozengurt, E. (2007) *J. Biol. Chem.* **282**, 10370–10379

33. Di Ciano, C., Nie, Z., Szasz, K., Lewis, A., Uruno, T., Zhan, X., Rotstein, O. D., Mak, A., and Kapus, A. (2002) *Ann. J. Physiol.* **283**, C850–C865

34. Dubé, N., Cheng, A., and Tremblay, M. L. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101**, 1834–1839

35. Head, J. A., Jiang, D., Li, M., Zorn, L. J., Schaefer, E. M., Parsons, J. T., and Weed, S. A. (2003) *Mol. Biol. Cell* **14**, 3216–3229