ACCELERATED PUBLICATION

This paper is available online at www.jbc.org

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 13, pp. 8313–8316, March 31, 2006

TSC1 Stabilizes TSC2 by Inhibiting the Interaction between TSC2 and the HERC1 Ubiquitin Ligase*[S]

Received for publication, November 21, 2005, and in revised form, February 3, 2006
Published, JBC Papers in Press, February 7, 2006, DOI 10.1074/jbc.C500451200

Huira Chong-Kopera[‡§¶1,2], Ken Inoki[‡1], Yong Li[‡1], Tianqing Zhu[‡], Francesc R. Garcia-Gonzalo[‖], Jose Luis Rosa[‖3], and Kun-Liang Guan[‡§¶4]

From the [‡]Life Sciences Institute, [§]Department of Biological Chemistry, and [¶]Institute of Gerontology, University of Michigan, Ann Arbor, Michigan 48109 and the [‖]Department de Ciències Fisiològiques II, IDIBELL, Campus de Bellvitge, Universitat de Barcelona, 08907 Barcelona, Spain

Tuberous sclerosis complex (TSC) is an autosomal dominant disease characterized by hamartoma formation in various organs. Two genes responsible for the disease, *TSC1* and *TSC2*, have been identified. The TSC1 and TSC2 proteins, also called hamartin and tuberin, respectively, have been shown to regulate cell growth through inhibition of the mammalian target of rapamycin pathway. TSC1 is known to stabilize TSC2 by forming a complex with TSC2, which is a GTPase-activating protein for the Rheb small GTPase. We have identified HERC1 as a TSC2-interacting protein. HERC1 is a 532-kDa protein with an E3 ubiquitin ligase homology to E6AP carboxyl terminus (HECT) domain. We observed that the interaction of TSC1 with TSC2 appears to exclude TSC2 from interacting with HERC1. Disease mutations in TSC2, which result in its destabilization, allow binding to HERC1 in the presence of TSC1. Our study reveals a potential molecular mechanism of how TSC1 stabilizes TSC2 by excluding the HERC1 ubiquitin ligase from the TSC2 complex. Furthermore, these data reveal a possible biochemical basis of how certain disease mutations inactivate TSC2.

Tuberous sclerosis complex (TSC)[5] is an autosomal dominant genetic disorder affecting 1 in 6,000–10,000 births (1). Mutations in either of the two genes, *TSC1* (also called hamartin) or *TSC2* (tuberin), cause the disorder characterized by benign tumor formation (hamartomas) in various organs and tissues. Complications of hamartomas in critical organs include renal failure, seizures, mental retardation, and autism (1). One of the hallmarks of TSC hamartomas is an increase in cell size, implicating TSC1 and TSC2 as negative regulators of cell growth (2–4).

Recent studies have revealed the molecular mechanism for the tumor suppressor function of TSC1 and TSC2, which form a physical and functional complex (5). The TSC1·TSC2 complex suppresses cell growth by inhibiting the mammalian target of rapamycin, mTOR, which is a central controller of cell growth. TSC1/TSC2 has GTPase-activating protein (GAP) activity toward the Rheb small GTPase (6, 7). Rheb acts upstream of and stimulates mTOR. TSC2 is the catalytic GAP subunit, while TSC1 enhances TSC2 function by stabilizing TSC2. The majority of disease-associated *TSC1* mutations identified result in no TSC1 protein being expressed; therefore, the free TSC2 protein in *TSC1* mutant cells is unstable (1). Similarly, many disease-derived TSC2 mutants are unstable due to weakened interaction with TSC1 (8, 9). However, the precise mechanism how TSC1 stabilizes TSC2 is largely unclear.

In this report, we identified HERC1 as a TSC2-interacting protein. The COOH-terminal region of HERC1 has a HECT E3 ubiquitin ligase domain (10). Interestingly, HERC1 does not associate with TSC1. Moreover, TSC1 efficiently competes with HERC1 for TSC2 binding. Our study provides a potential biochemical mechanism of TSC1 in TSC2 stabilization by inhibiting the interaction between TSC2 and the E3 ubiquitin ligase HERC1.

EXPERIMENTAL PROCEDURES

Plasmids and Antibodies—HA-TSC2 and Myc-TSC1 were described previously (8). Myc-HERC1 deletions were made by PCR cloning fragments into pRK5-Myc. HERC1 antibodies 363 and 410 were described previously (11). Commercial antibodies include anti-tuberin (Santa Cruz Biotechnology), anti-M2 FLAG (Sigma), anti-Myc 9E10, and anti-HA (Covance).

TSC2 Pull-down and Mass Spectrometry—One gram of brain tissue was homogenized in 5 ml of Nonidet P-40 lysis buffer. TSC2 protein complexes were immunoprecipitated by anti-TSC2. The immunoprecipitated samples were analyzed by silver stain and mass spectrometry analysis.

Co-immunoprecipitation Analysis—HEK293 cells were transfected with various plasmids. Cells were lysed in Nonidet P-40-lysis buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1% Nonidet P40, 50 mM NaF, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin) and immunoprecipitated with specific antibodies. Western blotting was performed to detect co-immunoprecipitated proteins.

For ubiquitination assay, cells were transfected with HA-TSC2 constructs and FLAG-Ubiquitin. 24 h after transfection, cells were treated with the proteosome inhibitor MG132 for 3 h prior to lysis. TSC2 proteins were immunoprecipitated, and ubiquitination was detected by anti-M2 FLAG antibody. For protein stability experiments, cultured cells were treated with cycloheximide (40 ng/ml) and chased for various time points.

RESULTS AND DISCUSSION

TSC2 Interacts with HERC1, a HECT Domain Containing Ubiquitin E3 Ligase—We sought to identify novel TSC2-interacting proteins. TSC2 antibody was incubated with mouse brain lysates, and immunocomplexes were visualized by SDS-PAGE and silver staining (Fig. 1*A*). The TSC2-interacting proteins were analyzed by mass spectrometry. One protein we identified was HERC1 (Fig. 1*A*); however, only peptides corresponding to the COOH-terminal half of the protein were found in the mass spectra (see supplemental Fig. S1). The mass spectrometry data are consistent with the observation that the HERC1 fragment migrated at ~200 kDa, whereas the full-length HERC1 is a very large protein of 532 kDa. HERC1 contains two regulator of chromosome condensation (RCC)-like domains (RLD), multiple WD40 repeats, and an E3 ligase HECT domain (Fig. 1*B*) (10, 11). Like TSC2, HERC1 is widely expressed in many tissues. The biological function of HERC1, however, has not been well defined.

To confirm the interaction between TSC2 and HERC1, we examined the association between transfected HA-TSC2 and endogenous HERC1 in HEK293 cells. Immunoprecipitation with an anti-HERC1 antibody revealed a specific interaction between HA-TSC2 and the endogenous HERC1 (Fig. 1*C*). In addition, we found that two HERC1-specific antibodies (denoted as 410 and 363) co-precipitated endogenous TSC2 (Fig. 1*C*). These data demonstrate that TSC2 and HERC1 interact with each other *in vivo*.

TSC1 Inhibits the Interaction between TSC2 and HERC1—To further define the interaction between TSC2 and HERC1, truncation mutants were constructed for HA-TSC2. Cells were co-transfected with the HA-TSC2 truncations and myc-HERC1-(3351-C). Myc-HERC1-(3351-C) was immunoprecipitated, and the presence of the TSC2 truncations was assessed by Western blot analysis. HERC1 was able to interact with COOH-terminal truncations of TSC2, suggesting that the GAP domain of TSC2 is not required for interaction with HERC1 (Fig. 2*A*). When only the NH_2-terminal third (1–608) of TSC2 was expressed, the TSC2 fragment was still able to bind to HERC1 though weaker than the other truncations (Fig. 2*A*). When the NH_2 terminus was deleted, TSC2-(400–1765) could not bind HERC1. Therefore, these data indicate that the NH_2-terminal domain of TSC2 is necessary and sufficient for HERC1 binding, although additional sequences outside of residues 1–608 in TSC2 may contribute to the HERC1 interaction. Deletion experi-

* This work was supported in part by grants from the National Institutes of Health (to K.-L. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.
[1] These authors made equal contributions.
[2] Supported by the National Institutes of Health Multidisciplinary Research Award T32-AG00114-18.
[3] Supported by grants from MEC (BFU-2004-06329/BMC) and the European Union (FEDER).
[4] To whom correspondence should be addressed. E-mail: kunliang@umich.edu.
[5] The abbreviations used are: TSC, tuberous sclerosis complex; HECT, homology to E6AP carboxyl terminus; GAP, GTPase-activating protein; RCC, regulator of chromosome condensation; RLD, RCC-like domain; HA, hemagglutinin; E3, ubiquitin-protein isopeptide ligase.

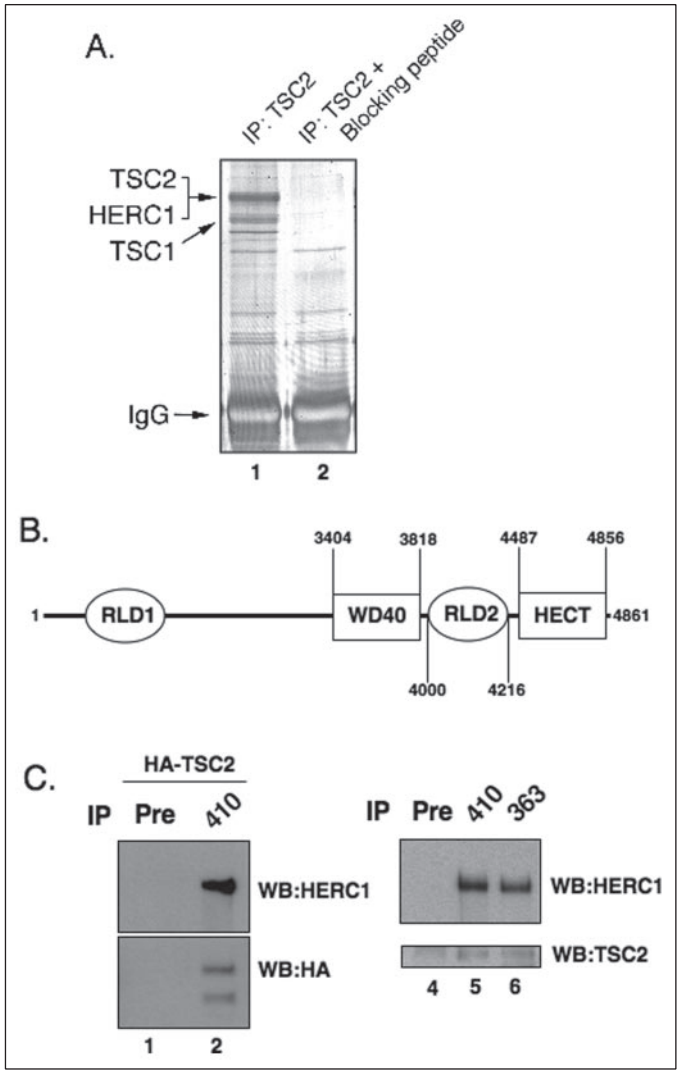

FIGURE 1. **HERC1 is a TSC2 interacting protein.** *A*, silver-stained gel of endogenous TSC2-associated proteins isolated from mouse brain lysates. HERC1 and TSC1 bands are shown. *B*, schematic diagram of HERC1. HERC1 contains two RLDs, a stretch of WD40 repeats, and a HECT domain. *C*, TSC2 binds HERC1 in HEK293 cells. HEK293 cells were transfected with HA-TSC2 as indicated (*lanes 1* and *2*). Lysates were immunoprecipitated with preimmune (*Pre*) or anti-HERC1 antibody (410). The immunoprecipitants were blotted with HERC1 (410) and HA antibodies. The lysate of untransfected HEK293 cells (*lanes 3–5*) was immunoprecipitated with preimmune serum (*Pre*) or two different anti-HERC1 antibodies (410, 363). Co-immunoprecipitated endogenous TSC2 were detected by anti-TSC2 antibody. IP and WB denote for immunoprecipitation and Western blot, respectively.

ments were also performed to determine the region of HERC1 responsible for interaction with TSC2. The data in Fig. 2*B* indicate that the COOH-terminal region of HERC1 contains at least two TSC2-interacting domains because both HERC1-(3001–3900) and -(3901-C) were co-precipitated with HA-TSC2. However, HERC1-(3001–3901) showed a weak interaction with TSC2. Therefore, it is possible that HERC1 contains a single TSC2-interacting domain around residue 3900.

It is interesting to note that the NH_2-terminal region of TSC2 is also responsible for interaction with TSC1 (12). Furthermore, it is well established that the ability to stabilize TSC2 may represent the most important function of TSC1 (5, 13). We, therefore, looked at the effect of TSC1 on the interaction between TSC2 and HERC1. The COOH-terminal domain of HERC1 (residues 3901-C) was co-expressed with TSC2 in the presence or absence of TSC1. TSC2 was able to complex with HERC1 in the absence of TSC1 co-transfection. However, when TSC1 was co-expressed, the TSC2-HERC1 interaction was completely inhibited (Fig. 2*C*, compare *lanes 2* and *3* with *4* and *5*). In contrast, the presence of HERC1 did not significantly affect the formation of the TSC1·TSC2 complex (Fig. 3*C*, compare *lanes 3* and *4*), suggesting that the HERC1-TSC2 interaction is weaker than the TSC1/TSC2 interaction. Nevertheless, the interaction between TSC2 and HERC1 is rather robust. It is worth noting that the expression levels of transfected TSC2 were only ~2–3 times higher than the endogenous TSC2 protein (Fig. 2*C*, *bottom panel*). The above data demonstrate that TSC1 binding to TSC2 excludes HERC1 from TSC2, and HERC1 only binds to the free TSC2.

Similar experiments were performed with HERC1-(3351-C). TSC1 also disrupted the interaction between TSC2 and HERC1-(3351-C) (see supplemental Fig. S2, *lanes 2–6*). Based on the above data, we propose that TSC1 stabilizes TSC2 by preventing the interaction between TSC2 and HERC1. Our model is consistent with the fact that both TSC1 and HERC1 bind to the NH_2-terminal region of TSC2.

TSC1 Fails to Disrupt the Interaction between HERC1 and the Unstable TSC2 Mutants Found in TSC Patients—We wanted to test whether HERC1 contributes to TSC2 destabilization. Down-regulation of HERC1 by RNA interference had little effect on TSC2 stability (data not shown). This is likely due to the presence of six HERC1 family members (29). HA-TSC2 was expressed in HEK293 cells alone or in the presence of HERC1-(3901-C). To determine TSC2 stability, cycloheximide was used to block protein synthesis. We observed that HERC1 co-transfection significantly decreased the stability of TSC2 (Fig. 3*A*). These observations support our model that HERC1 may contribute to TSC2 destabilization.

The majority of *TSC1* mutations that have been found in TSC patients result in no TSC1 protein production due to deletions or NH_2-terminal nonsense/frameshift mutations (1). In contrast, many disease-associated missense mutations of TSC2 show a weaker binding to TSC1 and are unstable (8, 9, 14). We co-transfected two TSC2 disease mutants with TSC1 and FLAG-ubiquitin and assessed the level of FLAG-ubiquitin on immunoprecipitated TSC2 proteins. The TSC2 R611Q and R905Q mutants were ubiquitinated at levels much higher than the wild type TSC2 (Fig. 3*B*). These disease mutants were previously shown to have weakened TSC1-binding (8). We then assessed whether the TSC2 disease mutants also bind HERC1 in the presence of TSC1. The disease mutants TSC2-R611Q and TSC2-R905Q both interacted with HERC1 as strong as the wild type TSC2 (Fig. 3*C*). The low intensity of mutant TSC2 in the HERC1 co-immunoprecipitation was due to lower expression levels of mutant TSC2 protein. Interestingly, TSC1 was not effective in inhibiting the interaction between TSC2-R611Q and HERC1 (Fig. 3*C*, *lanes 5–7*). Moreover, TSC1 had little effect on the interaction between TSC2-R905Q and HERC1 (Fig. 3*C*, *lanes 8–10*). These results are drastically different from the wild type TSC2, that TSC1 potently blocked the interaction between TSC2 and HERC1 (Fig. 3*C*, *lanes 2–4*). The above data reveal a possible biochemical basis of why the disease associated TSC2 mutants are unstable.

Our model predicts that TSC1 stabilizes TSC2 even in the presence of HERC1. We tested the effect of HERC1 expression on TSC2 stability when TSC1 was co-expressed. We found that HERC1 could not destabilize TSC2 if TSC1 was co-expressed (Fig. 3*D*, *lanes 1–5*). These results are consistent with the observation that TSC1 blocks the association between TSC2 and HERC1. We also examined the TSC2-R611Q mutant. As predicted, TSC2-R611Q was less stable than the wild type TSC2 even in the presence of TSC1 (Fig. 3*D*). Interestingly, expression of HERC1 further destabilized TSC2-R611Q even when TSC1 was co-expressed (Fig. 3*D*, *lanes 6–10*). It is worth noting that HERC1-(3901-C) is unstable, similar to many E3 ligases that have a short half-life. The differences of TSC2-R611Q stability in the presence or absence of HERC1 co-transfection were most dramatic at 1.5 and 3 h after cycloheximide treatment (Fig. 3*D*, *lanes 6–8*). Within this time range the co-transfected HERC1 protein was still present. These results indicate that HERC1 destabilizes TSC2 only when TSC1 is absent or TSC2 is not associated with TSC1.

The role of TSC2 in regulating the mTOR pathway has been intensely studied in recent years (8, 15–17). Clearly, the TSC proteins are key players in mTOR regulation and are involved in some of the most basic functions of cellular regulation, such as relaying signals from growth factors, nutrients, and energy levels to the cellular machinery that regulates cell growth and protein translation (2–4, 18). Genetic mutations in mouse and rat further confirm the tumor suppressor function of TSC1 and TSC2 (15, 19, 20). In *Drosophila*, mutation of TSC1 or TSC2 leads to a increase in cell size, demonstrating a key function of these proteins in cell growth and cell size regulation (2, 21).

The interaction between TSC1 and TSC2 results in protein stabilization and formation of a functional complex, in which TSC2 is the catalytic component to stimulate Rheb GTP hydrolysis (6, 7, 22, 23). In contrast, the apparent function of TSC1 is to bind and stabilize TSC2. This model explains why mutations in *TSC1* or *TSC2* produce almost identical phenotypes in *Drosophila* and mouse and why *TSC1* or *TSC2* mutations generate similar symptoms in TSC patients. In *TSC1*$^{-/-}$ cells, the TSC2 protein level is significantly lower

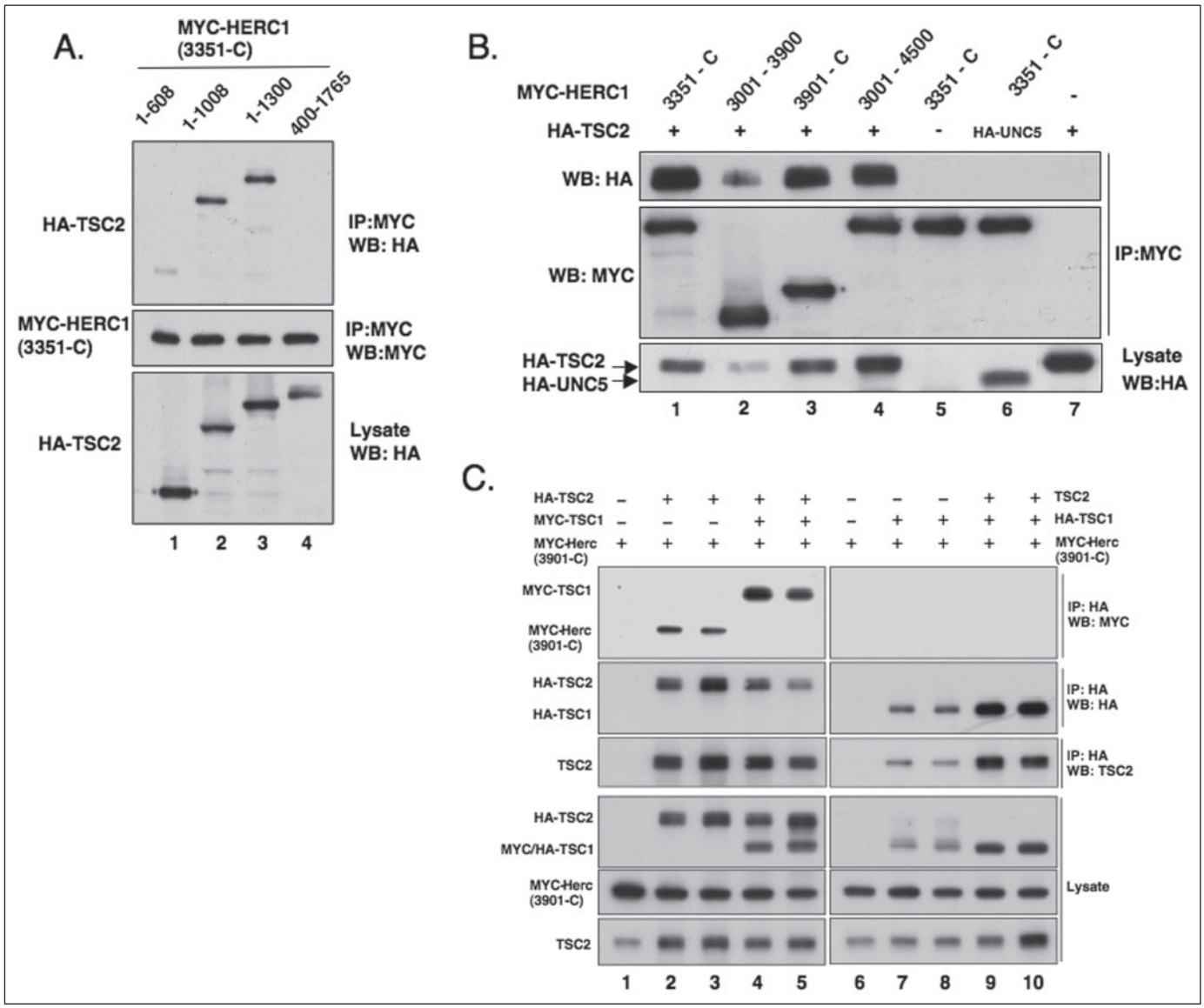

FIGURE 2. **TSC1 inhibits the interaction between TSC2 and HERC1.** *A*, the NH_2-terminal region of TSC2 is required for interaction with HERC1. Myc-HERC1-(3351-C) was co-transfected with various HA-TSC2 deletion constructs as indicated. Myc-HERC1 was immunoprecipitated, and the co-precipitated HA-TSC2 was detected by anti-HA Western blot. *B*, mapping the TSC2-interacting domains in HERC1. HA-TSC2 was co-transfected with various Myc-HERC1 deletion constructs as indicated. Myc-HERC1 was immunoprecipitated, and the co-precipitated TSC2 was detected by anti-HA antibody. HA-UNC5 was a negative control. *C*, TSC1 inhibits HERC1-(3901-C) from binding to TSC2. TSC2, HA-TSC2, HA-TSC1, Myc-TSC1, and Myc-HERC1-(3901-C) were transfected into HEK293 cells as indicated. Immunoprecipitation (*IP*) and Western blotting (*WB*) were indicated. 100 ng of Myc-HERC1-(3901-C) were transfected in all lanes. Because TSC1 and TSC2 stabilize each other, the DNA quantities used in the TSC1 and TSC2 co-transfection were less than those in the transfection of TSC1 or TSC2 alone. The transfected cDNA quantities (in ng) of TSC2 and TSC1 (TSC2/TSC1) plasmids were: 0/0 (*lane 1*), 250/0 (*lane 2*), 500/0 (*lane 3*), 100/100 (*lane 4*), 100/300 (*lane 5*), 0/0 (*lane 6*), 0/250 (*lane 7*), 0/500 (*lane 8*), 100/100 (*lane 9*), 300/100 (*lane 10*).

than in wild type cells (24). Several disease mutations in TSC2 weaken the interaction with TSC1 and decrease TSC2 stability, emphasizing the importance of a functional complex (8, 9, 14). However, the molecular mechanism of how TSC1 binding stabilizes TSC2 remained elusive.

Our study suggests a possible mechanism that TSC1 stabilizes TSC2 by inhibiting the interaction between TSC2 and HERC1; hence inhibiting TSC2 ubiquitination and degradation (see supplemental Fig. S3). Our data also provide a molecular basis for the instability of disease associated TSC2 mutations that can interact with HERC1 even in the presence of TSC1. Under normal conditions, HERC1 may have a limited effect on TSC2 stability regulation because TSC1 complexes with TSC2 and prevents the interaction between TSC2 and HERC1. Interestingly, phosphorylation of TSC2 by AKT in response to growth factor stimulation weakens its interaction with TSC1 and the phosphorylated TSC2 is unstable (8, 25). Therefore, it is possible that HERC1 may interact with and ubiquitinate the AKT-phosphorylated TSC2. Furthermore, HERC1 may also play an important role under some pathophysiological conditions, such as in *PTEN* mutant tumor cells that have high AKT activity. It is worth noting that HERC1 has been implicated in intracellular trafficking (10, 11). Currently, we have no data to exclude the possibility that HERC1 may regulate TSC2 trafficking through ubiquitination and, therefore, indirectly decrease TSC2 stability.

PAM, a ring finger-containing E3 ligase, was previously reported to physically and genetically interact with TSC2 (26). Mutation or deletion of *PAM* results in changes in cell size. However, a function of PAM toward TSC2 ubiquitination has not been shown. This may be due to the lack of an identified adaptor protein, which the E3 ligase Ring domain (27) in PAM may require for TSC2-specific ubiquitination. Both HERC1 and PAM are large proteins over 400 kDa that have an E3 ligase at the COOH terminus. Interestingly, HERC1 and PAM also contain RCC-like domains (RLD) (11, 26). RLD domains have been shown to stimulate nucleotide release of small GTPases such as Arf and Rab family proteins as well as Ran (11, 28). The formation of these large protein complexes may also suggest a scaffolding role of HERC1 and PAM. It has been reported that the human papilomavirus 16 E6 protein interacts with TSC2 and destabilizes TSC2 (30). TSC2 is a Rheb-specific GAP (6, 7, 22, 23). However, the identity of the Rheb GEF is not known; it is tempting to speculate that HERC1 may modulate Rheb activity by

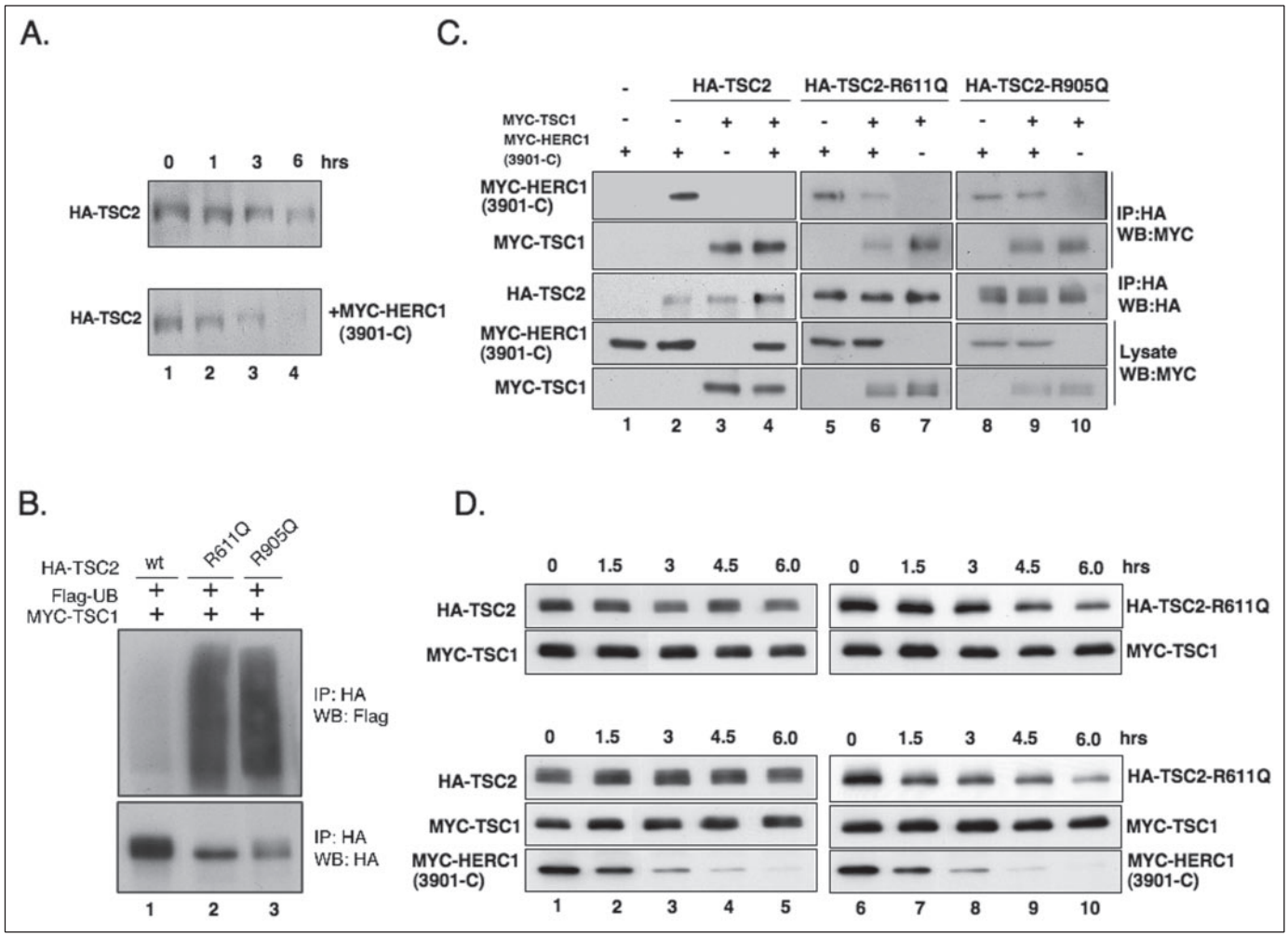

FIGURE 3. **TSC1 inhibits HERC1-induced TSC2 destabilization.** *A*, HERC1-(3901) destabilizes TSC2. HEK293 cells were co-transfected with HA-TSC2 alone (the *upper panel*) or HA-TSC2 and Myc-HERC1-(3901-C) (the *lower panel*). Cells were treated with cycloheximide for various times as indicated. *B*, disease-associated TSC-R611Q and R905Q mutants have increased ubiquitination. *C*, TSC1 is ineffective in preventing the interaction between HERC1 and TSC2-R611Q or TSC2-R905Q. Experiments were similar to Fig. 2*C*. *D*, TSC1 inhibits HERC1-induced destabilization of TSC2 but not TSC2-R611Q. Wild type TSC2 and TSC2-R611Q were in the *left* and *right panels*, respectively. Cycloheximide treatment (in hours) is indicated on the *top* of each lane.*IP* and *WB* denote for immunoprecipitation and Western blot, respectively.

acting as a GEF through its RLD domains. Future studies are required to test whether HERC1 could function as a Rheb GEF and to elucidate the role of HERC1 in regulation of cell growth and tumor development.

In summary, we have identified HERC1 as a novel TSC2 interacting protein. Our studies suggest a physiological function of HERC1 to regulate mTOR signaling by targeting TSC2 for degradation. This study also reveals a possible molecular mechanism of TSC1 in TSC2 stabilization, by which TSC1 complexes with TSC2 and prevents the association between TSC2 and the HERC1 ubiquitin ligase. Future studies are required to verify this model and to demonstrate the physiological functions of HERC1 in regulation of the TSC-mTOR pathway.

REFERENCES

1. Kwiatkowski, D. J. (2003) *Ann. Hum. Genet* **67,** 87–96
2. Pan, D., Dong, J., Zhang, Y., and Gao, X. (2004) *Trends Cell Biol.* **14,** 78–85
3. Hay, N., and Sonenberg, N. (2004) *Genes Dev.* **18,** 1926–1945
4. Fingar, D. C., and Blenis, J. (2004) *Oncogene* **23,** 3151–3171
5. van Slegtenhorst, M., Nellist, M., Nagelkerken, B., Cheadle, J., Snell, R., van den Ouweland, A., Reuser, A., Sampson, J., Halley, D., and van der Sluijs, P. (1998) *Hum. Mol. Genet* **7,** 1053–1057
6. Li, Y., Corradetti, M. N., Inoki, K., and Guan, K. L. (2004) *Trends Biochem. Sci.* **29,** 32–38
7. Manning, B. D., and Cantley, L. C. (2003) *Trends Biochem. Sci.* **28,** 573–576
8. Inoki, K., Li, Y., Zhu, T., Wu, J., and Guan, K. L. (2002) *Nat. Cell Biol.* **4,** 648–657
9. Nellist, M., Sancak, O., Goedbloed, M. A., Rohe, C., van Netten, D., Mayer, K., Tucker-Williams, A., van den Ouweland, A. M., and Halley, D. J. (2005) *Eur. J. Hum. Genet.* **13,** 59–68
10. Rosa, J. L., and Barbacid, M. (1997) *Oncogene* **15,** 1–6
11. Rosa, J. L., Casaroli-Marano, R. P., Buckler, A. J., Vilaro, S., and Barbacid, M. (1996) *EMBO J.* **15,** 4262–4273
12. Krymskaya, V. P. (2003) *Cell. Signal.* **15,** 729–739
13. Benvenuto, G., Li, S., Brown, S. J., Braverman, R., Vass, W. C., Cheadle, J. P., Halley, D. J., Sampson, J. R., Wienecke, R., and DeClue, J. E. (2000) *Oncogene* **19,** 6306–6316
14. Hodges, A. K., Li, S., Maynard, J., Parry, L., Braverman, R., Cheadle, J. P., DeClue, J. E., and Sampson, J. R. (2001) *Hum. Mol. Genet.* **10,** 2899–2905
15. Kwiatkowski, D. J., Zhang, H., Bandura, J. L., Heiberger, K. M., Glogauer, M., el-Hashemite, N., and Onda, H. (2002) *Hum. Mol. Genet* **11,** 525–534
16. Tee, A. R., Fingar, D. C., Manning, B. D., Kwiatkowski, D. J., Cantley, L. C., and Blenis, J. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 13571–13576
17. Gao, X., Zhang, Y., Arrazola, P., Hino, O., Kobayashi, T., Yeung, R. S., Ru, B., and Pan, D. (2002) *Nat. Cell Biol.* **4,** 699–704
18. Inoki, K., Ouyang, H., Li, Y., and Guan, K. L. (2005) *Microbiol. Mol. Biol. Rev.* **69,** 79–100
19. Yeung, R. S., Xiao, G. H., Jin, F., Lee, W. C., Testa, J. R., and Knudson, A. G. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 11413–11416
20. Onda, H., Lueck, A., Marks, P. W., Warren, H. B., and Kwiatkowski, D. J. (1999) *J. Clin. Invest.* **104,** 687–695
21. Thomas, G. (2002) *Biol. Res.* **35,** 305–313
22. Zhang, Y., Gao, X., Saucedo, L. J., Ru, B., Edgar, B. A., and Pan, D. (2003) *Nat. Cell Biol.* **5,** 578–581
23. Aspuria, P. J., and Tamanoi, F. (2004) *Cell. Signal.* **16,** 1105–1112
24. Zhang, H., Cicchetti, G., Onda, H., Koon, H. B., Asrican, K., Bajraszewski, N., Vazquez, F., Carpenter, C. L., and Kwiatkowski, D. J. (2003) *J. Clin. Invest.* **112,** 1223–1233
25. Potter, C. J., Pedraza, L. G., and Xu, T. (2002) *Nat. Cell Biol.* **4,** 658–665
26. Murthy, V., Han, S., Beauchamp, R. L., Smith, N., Haddad, L. A., Ito, N., and Ramesh, V. (2004) *J. Biol. Chem.* **279,** 1351–1358
27. Jackson, P. K., Eldridge, A. G., Freed, E., Furstenthal, L., Hsu, J. Y., Kaiser, B. K., and Reimann, J. D. (2000) *Trends Cell Biol.* **10,** 429–439
28. Bischoff, F. R., and Ponstingl, H. (1991) *Nature* **354,** 80–82
29. Garcia-Gonzalo, F. R., and Rosam, J. L. (2005) *Cell. Mol. Life Sci.* **62,** 1826–1838
30. Lu, Z., Hu, X., Li, Y., Zheng, L., Zhou, Y., Jiang, H., Ning, T., Basang, Z., Zhang, C., and Ke, Y. (2004) *J. Biol. Chem.* **279,** 35664–35670