HEC Binds to the Seventh Regulatory Subunit of the 26 S Proteasome and Modulates the Proteolysis of Mitotic Cyclins*

Yumay Chen, Z. Dave Sharp, and Wen-Hwa Lee‡

From the Department of Molecular Medicine and Institute of Biotechnology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78245-3207

A newly identified nuclear protein rich in leucine heptad repeats called HEC is important for mitosis. To elucidate its mechanism of action, the region containing leucine heptad repeats was used to identify cellular proteins that potentially interact with HEC. Complementary DNAs encoding several proteins including MSS1, p45, Nek2, and Smc1/Smc2, known to be important for G2/M progression, were identified. The interaction between HEC and MSS1, the seventh regulatory subunit of the 26 S proteasome, was further demonstrated by in vitro GST pull-down assays. HEC is not a part of the 26 S proteasome and interacts with MSS1 only when it is dissociated from the complex during M phase. Purified MSS1 specifically hydrolyzes ATP, an activity inhibited by HEC. In addition, HEC inhibits the proteolysis of mitotic cyclin B in vitro. Consistent with this biochemical activity, ectopic expression of HEC inhibits the degradation of mitotic cyclins after telophase, resulting eventually in cell death. These results show that HEC is an inhibitor of MSS1 and suggest that it may modulate M phase progression, in part, through the regulation of proteasome-mediated degradation of cell cycle regulatory proteins.

The ubiquitin-proteasome proteolytic pathway is important for a wide range of cellular functions including cell cycle control, DNA repair, peroxisome biogenesis, and resistance to heavy metals. In addition to recognizing and eliminating unassembled proteins and disposing of damaged or misfolded proteins, it is also responsible for the degradation of short-lived proteins, a process that is complex and highly regulated (reviewed in Refs. 1–3). The first step in this process is the ligation of the C terminus of ubiquitin to ϵ-amino groups of lysine residues within the substrate. After ubiquitination, the substrates are targeted to the 26 S proteasome complex, which contains over 20 distinct subunit activities, including multiple peptidases, ATPases, and a deubiquitinating enzyme. The 26 S proteasome is composed of two functionally distinct protein components, a catalytic 20 S proteasome and a regulatory complex. Controlled proteolysis requires that a regulatory complex be attached to each end of the 20 S proteasome. The regulatory complex is composed of 15 different subunits named according to their size as measured by SDS-polyacrylamide electrophoresis, with S1 being the largest and S15 the smallest. The proteolysis of certain substrates is ATP-dependent and requires polyubiquitination of the substrate.

Assembly of the 26 S proteasome is ATP-dependent, and the regulatory subunits have been found to belong to an ATPase subfamily that includes TBP-1, MSS1, p45, subunit 4 (mts2), CIM5, CIM3, and SUG1 (4–9). MSS1, first identified as a co-activator of TAT-mediated transactivation (10, 11), was later found to be the seventh regulatory subunit of the 26 S proteasome complex (5). Temperature-sensitive mutants of CIM5, the homologue of human MSS1 in S. cerevisiae, progress through the cell cycle normally at permissive temperatures. At restrictive temperatures, they arrest at the G2/M boundary coincident with the loss of 26 S proteasome activity (8). These observations linked 26 S proteasome activity directly to cell cycle control, specifically during the mitotic phase.

In metazoan cells, cyclin degradation by regulated proteolysis is crucial for M phase progression (reviewed in Refs. 1 and 12). In this stage of the cell cycle, a large protein complex called the anaphase-promoting complex (APC,† Ref. 13) starts chromosome segregation and exit from mitosis through targeting anaphase inhibitors and mitotic cyclins for degradation by way of the 26 S proteasome. Although interdependent, this pathway is distinct from the proteolytic CDC34 pathway that participates in the regulated proteolysis of the G1 cyclins (12). Recognition of the APC substrates for ubiquitination is by way of a 9-amino acid motif referred to as the destruction (D) box. A key event in anaphase progression in yeast is the APC-mediated proteolysis of a non-cyclin substrate (CUT2, fission yeast; PSD1, budding yeast) that is an inhibitor of anaphase initiation (14, 15). Although much is known concerning the identity of the players in APC-mediated ubiquitination, little is understood regarding their regulation or the control of 26 S proteasomes. Nevertheless, it is clear that regulation of proteolysis in M phase is key to its orderly progression.

The retinoblastoma protein (Rb) has an important role in regulating the cell cycle as well as differentiation, development, and the suppression of malignant transformation (16). The isolation of cellular proteins that interact with Rb has provided important information regarding the molecular basis of its function (17–19). One of these interacting proteins (18), originally identified as a partial cDNA, C15, was later named HEC because of its high expression in cancers. During its characterization, HEC was found to be a centromere-associated protein with a critical role in chromosome segregation. When HEC is deregulated in cells by microinjection with specific antibodies, abnormal daughter cells are produced that fail to

---

* This work was supported by National Institutes of Health Grants EY05758 and CA58318 and a grant from the Council for Tobacco Research. 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.

† To whom correspondence should be addressed: Dept. of Molecular Medicine and Institute of Biotechnology, University of Texas Health Science Center at San Antonio, 15355 Lambda Dr., San Antonio, TX 78245-3207. Tel.: 210-567-7353; Fax: 210-567-7377; E-mail: leew@uthscsa.edu.

1 The abbreviations used are: APC, anaphase-promoting complex; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; GFP, green fluorescence protein; DAPI, 4’,6-diamidino-2-phenylindole.
HEC Modulates Mitosis

proceed to the next division and subsequently die (20).

To elucidate the potential role of HEC in M phase progression, the cellular proteins interacting with HEC through its long stretch of leucine heptad repeats were identified using yeast two-hybrid screening. Interestingly, one of these proteins had been previously identified as MSS1, the seventh regulatory subunit of the 26 S proteasome. The ATPas enzyme of MSS1 is down-regulated by HEC, and degradation of cyclin B by proteasomes is prevented by the addition of HEC in an in vitro assay. Furthermore, ectopic expression of a HEC mutant inhibits mitotic cyclin degradation during mitosis in vivo. These results suggest that the interaction between HEC and MSS1 is biologically significant.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screen for HEC-associated Proteins—The HEC protein has two major domains: an amino-terminal portion containing a region that interacts with the Rb protein and a carboxyl-terminal region that is enriched with heptad leucine repeats (20). The C-terminal half of the HEC cDNA encoding the leucine repeat-rich region (amino acids 251–618) was ligated into the modified yeast vector, pAS1, and used as bait in a yeast two-hybrid screen of a human lymphocyte cDNA library using previously published procedures (17, 18). Among the 16 strongly interacting clones obtained, 12 were published (26). Cell lysates prepared from differentially synchronized populations of T24 cells were loaded on 5–25% preformed sucrose gradients and centrifuged in an SW 50.1 rotor (Beckman, Fullerton, CA) at 28,000 rpm for 15 h at 4 °C. Aliquots of each fraction were then assayed for the presence of MSS1 and HEC by Western blotting analysis as described above.

Transfections—Three constructs were used in transfection assays: 1) CHPL-GFP, a modified plasmid derived from a mammalian expression vector containing a Myc-tagged, mutant form of green fluorescence protein (S65T; Ref. 27) (CLONTECH); 2) CHPL-GFP-I5P, containing GFP fused to the N terminus of HEC (amino acids 1–250); and 3) CHPL-GFP-I5Pst, containing GFP fused to the C-terminal portion of HEC (amino acids 251–618). Transfection of 1 × 10⁶ cells was done using a conventional calcium phosphate/DNA-co precipitation method (17). The precipitates were removed 12 h after transfection, and the cells cultured with fresh medium. The cells were observed under a fluorescence microscope (Axiopt Photomicroscope, Zeiss).

Immunostaining—Cells grown on coverslips in tissue culture dishes were washed in phosphate-buffered saline (PBS) and fixed for 30 min in 4% formaldehyde in PBS with 0.5% Triton X-100. After treatment with 0.05% saponin in water for 30 min and extensive washing with PBS, cells were blocked in PBS containing 10% normal goat serum. An overnight incubation with antibody diluted in 1% bovine serum albumin, and 10 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, and 2 mM dithiothreitol, 50 µg/ml bovine serum albumin, and 10 mM ATP. Reactions were stopped by the addition of EDTA to 50 mM. 2 µl of the reactions were spotted onto polyethyleneimine TLC plates (Sigma) and developed in 0.5 × LCI, 1 M formic acid (22). The amount of released inorganic phosphate was quantified using a PhosphorImager analysis. For the antibody blocking experiment, 1 µl of mouse polyclonal anti-C15 antisera or 1 µg of anti-Myc 9E10 (25), were added to each clarified supernatant. After a 1-h incubation, protein A-Sepharose beads were added for another hour. The beads were then collected and washed five times with lysis buffer containing 250 mM NaCl and then boiled in SDS-loading buffer for immunoblotting analysis as described (23). Anti-green fluorescence protein (anti-GFP) (CLONTECH, Palo Alto, CA) was used at 1:250 dilution for immunoblotting analysis.

Fractionation by Sucrose Gradient Centrifugation—The procedures to fractionate cell components were adapted from those previously published (26). Cell lysates prepared from synchronously labeled cultures of T24 cells were loaded on 5–25% preformed sucrose gradients and centrifuged in an SW 50.1 rotor (Beckman, Fullerton, CA) at 28,000 rpm for 15 h at 4 °C. Aliquots of each fraction were then assayed for the presence of MSS1 and HEC by Western blotting analysis as described above.

RESULTS

HEC Interacts with Cellular Proteins That Have Critical Roles in Mitosis—To explore the potential biochemical basis for the biological function of HEC, we sought to obtain clues by searching for proteins with which it interacts. Since leucine heptad repeats are known to be important in protein–protein interactions (28), the C-terminal two-thirds of HEC containing the long stretches of leucine repeats was used as bait in a yeast two-hybrid screen of a human lymphocyte cDNA library. Among the 16 strongly interacting clones obtained, 12 were identified as cDNA fragments encoding MSS1, a component of subunit 7 of the 26 S proteasome complex (5, 10). Others obtained encoded subunits 3, 5, and 6 of the 26 S proteasome (6); Sb18, 10% fetal calf serum, were synchronized at G1, by density arrest in Dulbecco’s modified Eagle’s medium, 0.5% serum and then released at time 0 by replating in Dulbecco’s modified Eagle’s medium, 10% fetal calf serum at a density of 2 × 10⁵ cells/cm². At various time points thereafter (18 h for G₂/S, 22 h for S, 33 h for G₁), cells were harvested. To obtain cells in M phase, nocodazole was added to the culture medium for 8 h prior to harvest as described previously (23).

Immuno precipitation and Western Blot Analysis—Cells lysed in Lysis 250 buffer were subjected to three freeze/thaw cycles (liquid nitrogen/37 °C), and clarified by centrifugation (10,000 × g, 2 min at room temperature). The supernatants were used for immunoprecipitation as described (24). Briefly, 1 µl of mouse polyclonal anti-C15 antisera or 1 µg of anti-Myc 9E10 (25), were added to each clarified supernatant. After a 1-h incubation, protein A-Sepharose beads were added for another hour. The beads were then collected and washed five times with lysis buffer containing 250 mM NaCl and then boiled in SDS-loading buffer for immunoblotting analysis as described (23). Anti-green fluorescence protein (anti-GFP) (CLONTECH, Palo Alto, CA) was used at 1:250 dilution for immunoblotting analysis.
Hatched domain.

autoradiography.
sample for 30 min at room temperature. Following extensive washing, the complexes were separated by SDS-PAGE, dried, and visualized by

grown in liquid cultures and used for ONPG quantitation of

transactivation domain fusion protein and used to test for interaction with HEC fusion proteins in yeast two-hybrid assays. Transformants were

protein were used for the binding reactions indicated

proteins were washed extensively, and the samples were quantitated by Coomassie Blue staining of SDS-PAGE gels. Equivalent amounts of

GST-MSS1

full-length MSS1 (amino acids 328–434) was used in the yeast two-hybrid system to assay for their ability to

interact with MSS1. As shown in (Fig. 1

binding experiment was performed using a

in vitro

lysate transcription-translation system and tested for its abil-

HEC was synthesized by a reticulocyte

S glutathione S-transferase (GST) and in-frame GST fusions with cDNAs encoding full-length MSS1 (GST-MSS1) and C-terminal amino acids 328–434 of MSS1 (GST-MSS1C) were expressed in E. coli. GST and GST fusion proteins were washed extensively, and the samples were quantitated by Coomassie Blue staining of SDS-PAGE gels. Equivalent amounts of protein were used for the binding reactions indicated above each lane. In vitro translated (IVT) full-length HEC (lane 1) was mixed with the bound sample for 30 min at room temperature. Following extensive washing, the complexes were separated by SDS-PAGE, dried, and visualized by autoradiography. B, complementary DNAs encoding deletion mutants of HEC were generated by in-frame fusion to the GAL4 DNA-binding domain. Hatched, shadetied, and stippled regions are the three leucine heptad repeat-rich domains in HEC. MSS1 was expressed as a GAL4 transactivation domain fusion protein and used to test for interaction with HEC fusion proteins in yeast two-hybrid assays. Transformants were grown in liquid cultures and used for ONPG quantitation of β-galactosidase activity. The -fold increase in the activities compared with the host yeast strain, Y153, are indicated. Assays were done in triplicate for each transformation.

the human homologue of yeast Smc1/Smc2 (29–31); and Nek2 (32), the human homologue of NimA (33), a kinase crucial for the progression of G2/M phase in Aspergillus nidulans (Table I).

**HEC Interacts Specifically with MSS1**—The importance of regulated proteolysis in the cell cycle (8) and, in particular, chromosome condensation during M phase (1, 12) is well estab-

lished. To further assess the biological significance of the asso-

ciation between HEC and its interacting proteins, we chose to focus first on MSS1. To further confirm the yeast two-hybrid results, an in vitro binding experiment was performed using a GST pull-down assay. HEC was synthesized by a reticulocyte lysate transcription-translation system and tested for its ability to bind GST-MSS1 and GST-MSS1C (amino acids 328–434) fusion proteins. The results showed that the C-terminal portion of MSS1 is sufficient for binding to HEC (Fig. 1A). To determine the region(s) of HEC necessary for an interaction with MSS1, three HEC deletion constructs, each containing a variable number of the leucine heptad repeats, were fused in frame to the GAL4 DNA-binding domain. These constructs were then used in the yeast two-hybrid system to assay for their ability to interact with MSS1. As shown in (Fig. 1B), the second region of leucine heptad repeats located between amino acids 361 and 547 of HEC is sufficient for interactions with MSS1.

**MSS1, but Not HEC, Is a Component of the 26 S Proteasome**—MSS1 is the seventh regulatory subunit of the 26 S proteasome complex (5). Since it binds to MSS1 in vitro, HEC could also be a constituent of the regulatory complex of the 26 S proteasome. To test this possibility, we obtained three active fractions of partially purified 26 S proteasomes (kindly provided by M. Rechsteiner; see Fig. 2, lanes 2–4) and assayed for

the presence of MSS1 and HEC. Consistent with previous sequencing data from subunit 7 peptides isolated from 26 S proteasomes (5), MSS1 was detected in all three fractions containing 26 S proteasome activity (Fig. 2, lanes 6–8), while HEC was not detected in any of the fractions (Fig. 2, lanes 10–12). However, MSS1 and HEC are both detected in total cellular protein lysate (Fig. 2, lanes 5 and 9). This result suggests that HEC is not present in the 26 S proteasome complex.

**Cell Cycle-dependent Association between HEC and MSS1**—Since HEC is not a component of the 26 S proteasome but does interact with the MSS1, a regulatory subunit of the protea-

some, it is important to determine the temporal and spatial status of these two proteins in cells. To answer these questions, specific antibodies were used to examine expression during cell cycle progression. HEC is expressed most abundantly during late S to M phase (Fig. 3B, top panel), while MSS1 is expressed throughout the cell cycle (Fig. 3A, middle panel). Co-immunoprecipitation of HEC and MSS1 was detected only during M phase (Fig. 3B, middle panel). To further assess the potential of a HEC/MSS1 interaction, a sedimentation analysis of cellular lysates was performed. In cell lysates prepared from cells synchronized in S phase, the majority of MSS1 was found to be in fractions consistent with its association with the 26 S protea-

some complex (Fig. 4A). This fraction is distinct from that containing HEC, which, during this phase, sediments at a lower S value (Fig. 4A). In M phase-synchronized cells, a significant portion of MSS1 co-sediments with HEC in the lower S value fractions (Fig. 4B). These data are consistent with the results in Fig. 3B, which showed that HEC/MSS1 co-immunoprecipitated only during M phase.

**HEC Represses the ATPase Activity of MSS1**—A potential

### Table I

| HEC-Aps clones | Binding in yeast<sup>a</sup> | In vitro binding | Identity | Homologue | Mutant phenotype |
|----------------|---------------------------|-----------------|----------|-----------|-----------------|
| 1, 6, 7, 8<sup>b</sup> | 371.5 ± 19.3 | + | MSS1 | CIM5 | G/M arrest |
| 4 | 273.3 ± 10.0 | + | Smc1/Smc2 | | |
| 14 | 239.4 ± 32.6 | + | Nima | | |
| 24 | 1105.2 ± 159.3 | + | p45 subunit of 26 S proteasome | CIM5 (SUG1) | G/M arrest |

<sup>a</sup> β-Galactosidase activity.

<sup>b</sup> Total number obtained was 12.
function of the HEC/MSS1 interaction is to regulate proteasome activity by modulation of MSS1. MSS1, as well as several other cloned regulatory subunits of the 26 S proteasome (S4, TBP1, SUG1, CIM5, CIM3, and p45), contain ATPase domains (4). Presumably, the putative ATPase activity common to all of these regulatory subunits is an important aspect of their function. Alterations in this activity could be important for regulation of proteasome function. Although MSS1 is presumed to be an ATPase, this activity, to our knowledge, has never been addressed the possibility that HEC may regulate the degradation of mitotic cyclins. As shown in Fig. 7, cyclin B synthesized by rabbit reticulocyte lysates can be degraded in vitro using a proteasome degradation assay. The addition of GST had no influence on cyclin B degradation, while the addition of GST-HEC or GST-15Pst significantly inhibited cyclin B degradation. These results suggest that HEC may down-regulate proteasome activity.

**HEC Can Inhibit Cyclin B Degradation in Vitro**—Since HEC association with MSS1 is only detectable during M phase of the cell cycle and because it can negatively regulate the ATPase activity of MSS1, we next investigated the possibility that HEC can regulate the degradation of mitotic cyclins. As shown in Fig. 7, cyclin B synthesized by rabbit reticulocyte lysates can be degraded in vitro using a proteasome degradation assay. The addition of GST had no influence on cyclin B degradation, while the addition of GST-HEC or GST-15Pst significantly inhibited cyclin B degradation. These results suggest that HEC may down-regulate proteasome activity.

**Ectopic Expression of the Leucine Heptad Repeats of HEC Interferes with Mitotic Cyclin Degradation**—We next wished to address the possibility that HEC may regulate the degradation
of mitotic cyclins in cells. This was hampered by the deleterious effects of HEC overexpression in cells. An alternative approach to circumvent this problem was to express a truncated form of HEC lacking an NLS. It was postulated that, under conditions of cytoplasmic accumulation of HEC, it should be possible to observe the effects after nuclear envelope breakdown. Since the heptad repeats in HEC that bind to MSS1 were sufficient for inhibition of MSS1 activity in vitro, we sought to determine whether expression of this region of HEC alone, which lacks the NLS, could influence cell division and/or mitotic cyclin degradation. Two constructs using two separate regions of HEC fused to GFP were used; GFP-15PA contains the N-terminal region only (amino acids 1–250), and GFP-15Pst contains the entire series of leucine heptad repeats (amino acids 251–618) (Fig. 8A). The GFP parent vector served as a control. Transfection of these three constructs into Rb-negative Saos-2 cells resulted in expression of the corresponding proteins, which could be detected by Western blotting using anti-GFP and anti-Myc tag antibodies as probes (Fig. 8B, lanes 2–4).

Twenty-four hours after transfection, cells were observed directly using fluorescence microscopy. Expression of GFP was detected in nuclei and cytoplasm (Fig. 8C, part b), while GFP-15PA was observed only in the nuclei (Fig. 8C, part d) and GFP-15Pst was found only in the cytoplasm (Fig. 8C, part f), consistent with loss of the potential NLS. In contrast to HEC inactivation with anti-HEC antibodies, no abnormal daughter cells were observed under conditions of HEC cytoplasmic accumulation. However, when these cells were immunostained with antibodies specifically recognizing cyclin A or cyclin B, neither cyclin A (Fig. 8D, part c, arrowhead) nor cyclin B (Fig. 8E, part c, arrowhead) could be detected in the untransfected cells after telophase. In contrast, in pre- or metaphase cells, both cyclin A and B were detectable (Fig. 8, D and E, labeled with asterisk).

**Fig. 5.** MSS1 contains ATPase activity. A, purity of the GST fusion proteins. GST-MSS1 (lane 1), GST-HEC (lane 2) and GST-15Pst (lane 3) were expressed in E. coli, and the fusion proteins were purified by glutathione-Sepharose column. After extensive washing, the purity of the fusion proteins were examined by Coomassie Blue staining of SDS-PAGE gels. B, ATPase activity of MSS1. The ATPase activity of MSS1 was determined as described under “Experimental Procedures.” MSS1 and HEC fused to GST in the indicated amounts were used in the assays. C, antibodies to MSS1 inhibit ATPase activity. Antibodies to either MSS1 or GST were included in the assays as indicated. D, nucleoside specificity of MSS1 ATPase. In addition to ATP, either GTP, CTP, or UTP was used in the assays in the amounts indicated.

**Fig. 6.** HEC can repress MSS1’s ATPase activity. ATPase activity was determined as described under “Experimental Procedures.” The results using the indicated GST-HEC fusions (see Fig. 1) and GST in the indicated amounts are shown.

**Fig. 7.** HEC inhibits cyclin B degradation in vitro. Cyclin B was synthesized in a TNT transcription and translation coupled system. In each reaction, the same amount of cyclin B with identical concentration of GST or GST-HEC and GST-15Pst was incubated at 30 °C for the time indicated. The remaining cyclin was quantitated by PhosphorImager and plotted against time.
In cells expressing GFP-15Pst, cyclin A (Fig. 8D, part f, arrow) and cyclin B (Fig. 8E, part f, arrow) were detected even after telophase. These results strongly suggest that overexpression of the C-terminal region of HEC can inhibit the degradation of mitotic cyclins during M phase.

**DISCUSSION**

Previous studies suggest that HEC is essential for normal mitosis in mammalian cells (20). Cells injected with antibodies specific to HEC demonstrate multiple abnormalities with regard to mitosis: 1) chromosomes condense but fail to segregate properly; 2) no metaphase plates are observed; 3) spindles are disorganized in relation to the centromeres; 4) spindles fail to assume the proper orthogonal orientation to chromatids; and 5) cells are able to cytokinese, but chromosomes are separated haphazardly into abnormal, nonviable daughter cells. These studies point to an important role of HEC in cell division.

Inactivation of HEC apparently disrupts the delicate regulation of early M phase events. Since a common phenotype of HEC inactivation is a disorganized metaphase plate, HEC action probably begins early in mitosis, perhaps during prophase. The potential substrates for regulating this part of M phase remain to be elucidated.

The localization of a portion of HEC at the centromere/kinetochore (20) indicates that the protein may be involved in spindle attachment to chromosomes during prophase and indirectly involved in subsequent chromosome movement (34). However, the lack of a signature tubulin-binding domain in the HEC molecule argues against direct microtubule attachment.

The association of HEC with a mitosis-specific kinase and with several subunits of the 26 S proteasome (Table I) suggests potential ways by which HEC may influence chromosome congression, separation, or segregation. In this role, HEC may function as an adaptor molecule through its long leucine heptad repeats, much like the Skp1 protein in budding yeast (35, 36). HEC may alter the conformation of multiple-subunit complexes and bring together a number of proteins, including components of the mitotic spindle or kinetochore, components of the 26 S proteasome, kinases or phosphatases, and checkpoint monitors. Regulatory events during chromosome alignment and separation are rapid and precisely timed, and they are likely to be profoundly disturbed without appropriate coordinating adaptor molecules. This concept is consistent with the recent finding that the APC, composed of at least seven distinct proteins, is required for both chromosome segregation and exit from mitosis (37, 38).

The dynamics of the association of MSS1 with different complexes are clear from the results presented here. HEC is not a component of the 26 S proteasome. It is, however, found in association with MSS1 as part of a smaller complex that appears in the late S to M phase. One explanation of how HEC might regulate mitotic cyclin degradation is that it binds to MSS1 and somehow inhibits its assembly into the 26 S proteasome. Alternatively, HEC may serve as a link between E3/APC cyclosome (12) and the 26 S proteasome to mediate substrate specificity for controlled proteolysis.

---

**FIG. 8. Expression of a HEC deletion mutant interferes with mitosis.** A, schematic diagram of full-length HEC, GFP-15PA (containing only amino acids 1–250), and GFP-15Pst (encoding amino acids 251–618, encompassing the entire leucine heptad repeat domain). B, detection of GFP and GFP-HEC fusion proteins in transfected Saos-2 cells. After transient transfection, cell lysates were separated by SDS-PAGE. Expression of GFP fusion proteins was determined by immunoprecipitation with anti-Myc I–9E10 mAb (24), followed by blotting with anti-GFP antibody. GFP (lane 1), GFP-15PA (lane 2), and GFP-15Pst (lane 3) fusion proteins are indicated by asterisks. The arrow marks the IgG heavy chain. C, localization of GFP and GFP-HEC fusion proteins in Saos-2 cells. Phase contrast images (a, c, and e) and GFP autofluorescence (b, d, and f) show the subcellular location of the various GFP-HEC fusion proteins. D, perturbation of cyclin A levels in mitotic cells that expressed GFP-15Pst. DAPI (a and d) identifies DNA in nuclei; GFP autofluorescence (b and e) tagged the cells that expressed GFP-HEC fusion proteins; and indirect immunofluorescence with anti-cyclin B primary antibodies and Texas Red-labeled secondary antibodies shows the location of cyclin B (c and f). E, disturbance of cyclin B levels in cells that expressed GFP-15Pst. DAPI (a and d) identifies DNA in nuclei; GFP autofluorescence (b and e) shows the cell that expressed GFP-HEC fusion proteins; and indirect immunofluorescence with anti-cyclin B primary antibodies and Texas Red-labeled secondary antibodies show the location of cyclin B (c and f). Note that cyclin A (panel D, parts d–f) and cyclin B (panel E, parts d–f) are present in telophase cells when GFP-15Pst is overexpressed.
In previous experiments, HEC inactivation by antibody injection leads to abnormal mitosis that includes many obvious defects discussed above. Overexpressing a truncated, cytoplasmic form of HEC does not produce exactly the same phenotype in cells. However, the common phenotype observed under conditions of antibody injection and mutant overexpression is that cells progress through cytokinesis, but daughter cells fail to enter the next division and die. Apparently, mutant HEC lacks a dominant negative effect and may fail to form dimers with the wild-type HEC. Since HEC is found in association with a portion of MSS1 separate from the 26 S proteasome specifically in M phase, it is likely that the overexpressed mutant HEC binds to MSS1 during the time when the nuclear membrane breaks down. Consistent with its negative influence on cyclin B degradation in vitro, overexpression of this mutant HEC did result in an abnormal nuclear accumulation of cyclin A and B in cells as late as telophase. Although the precise mechanism for this phenotype is yet to be elucidated, it is likely that mutated HEC disrupts the dynamics of MSS1 availability for 26 S proteasome assembly. In this regard, the N-terminal region of HEC may have important regulatory elements for HEC function. Indeed, it is known to contain at least three recognizable elements: a putative NLS, a Nek2 phosphorylation site (39), and a region for specific interaction with Rb. These elements may provide an additional mechanism for regulating the quantity and/or quality of HEC during normal progression of the cell cycle.

Our working model envisions HEC as an adaptor that inhibits the degradation of mitotic cyclins, thus allowing their accumulation during early M phase. Upon receipt of an unidentified signal, HEC repression is relieved, M phase cyclins are degraded, sister chromatid separation ensues, and cell division is completed. This model as well as the consequence of other HEC interactions are currently under investigation to more fully understand the possible role of HEC in mitosis.

REFERENCES

1. Ciechanover, A. (1994) Cell 79, 13–21
2. Tanaka, K. (1995) Mol. Biol. Rep. 21, 21–26
3. Dubiel, W., Ferrell, K., and Rechsteiner, M. (1995) Mol. Biol. Rep. 21, 27–34
4. Nelbock, P., Dillon, P. J., Perkins, A., and Rosen, C. A. (1996) Science 248, 1650–1653
5. Dubiel, W., Ferrell, K., and Rechsteiner, M. (1993) FEBS Lett. 323, 276–278
6. Akiyama, K., Yokota, K., Kagawa, S., Shimbara, N., DeMartino, G. N., Slaughter, C. A., Noda, C., and Tanaka, K. (1995) FEBS Lett. 363, 151–156

---

2 Y. Chen and W.-H. Lee, unpublished observations.
HEC Binds to the Seventh Regulatory Subunit of the 26 S Proteasome and Modulates the Proteolysis of Mitotic Cyclins
Yumay Chen, Z. Dave Sharp and Wen-Hwa Lee

J. Biol. Chem. 1997, 272:24081-24087.
doi: 10.1074/jbc.272.38.24081

Access the most updated version of this article at http://www.jbc.org/content/272/38/24081

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 39 references, 22 of which can be accessed free at http://www.jbc.org/content/272/38/24081.full.html#ref-list-1