



**HAL**  
open science

## The mRNA Nuclear Export Factor Hpr1 Is Regulated by Rsp5-mediated Ubiquitylation

Carole Gwizdek, Maria Hobeika, Bart Kus, Batool Ossareh-Nazari, Catherine Dargemont, Manuel Rodriguez

► **To cite this version:**

Carole Gwizdek, Maria Hobeika, Bart Kus, Batool Ossareh-Nazari, Catherine Dargemont, et al..  
The mRNA Nuclear Export Factor Hpr1 Is Regulated by Rsp5-mediated Ubiquitylation. *Journal of Biological Chemistry*, 2005, 280 (14), pp.13401-13405. 10.1074/jbc.C500040200 . hal-03084499

**HAL Id: hal-03084499**

**<https://hal.science/hal-03084499>**

Submitted on 21 Dec 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## The mRNA Nuclear Export Factor Hpr1 Is Regulated by Rsp5-mediated Ubiquitylation\*

Received for publication, January 28, 2005, and in revised form, February 14, 2005  
Published, JBC Papers in Press, February 15, 2005, DOI 10.1074/jbc.C500040200

Carole Gwizdek<sup>‡§¶</sup>, Maria Hobeika<sup>‡§¶</sup>, Bart Kus<sup>\*\*</sup>, Batool Ossareh-Nazari<sup>‡</sup>,  
Catherine Dargemont<sup>‡ ¶‡</sup>, and Manuel S. Rodriguez<sup>‡</sup>

From the <sup>‡</sup>Institut Jacques Monod, Unité Mixte de Recherche 7592, CNRS, Universités Paris VI and VII, 75251 Paris Cedex 05, France and the <sup>\*\*</sup>University of Toronto, Ontario M5G 1L6, Canada

Ubiquitin conjugation and in particular two distinct HECT ubiquitin ligases, Rsp5p and Tom1p, have been shown to participate in the regulation of mRNA export in *Saccharomyces cerevisiae*. The identification of the ubiquitin ligase substrates represents a major challenge in understanding how this modification may modulate mRNA export. Here, we identified Hpr1p, a member of the THO/TREX (transcription/export) complex that couples mRNA transcription to nuclear export as a target of the ubiquitin-proteasome pathway. Hpr1p degradation is enhanced at high temperature and appears linked to on-going RNA-polymeraseII-mediated transcription. Interestingly, the stability of the other THO complex components is not affected under these conditions indicating that Hpr1p turnover could control the formation of the THO/TREX complex and consequently mRNA export. Using *in vivo* and *in vitro* approaches we demonstrate that Rsp5p is responsible for the ubiquitylation of Hpr1p that also involves the ubiquitin-conjugating enzyme Ubc4p. Thus, Hpr1p represents the first nuclear export factor regulated by ubiquitylation, strongly suggesting that this post-translational modification participates in the coordination of transcription and mRNA export processes.

In eukaryotic cells, intracellular transport pathways are modulated as a function of differentiation, development, and cell cycle in response to a variety of stimuli. However, the molecular mechanisms ensuring this plasticity remain poorly characterized and probably affect both transport machineries and cargoes. Obviously, post-translational modifications and in particular ubiquitylation play a key role in control and regulation of intracellular transport. Indeed, the initial interest of ubiquitylation was focused on the role of polyubiquitin chains in targeting proteins to degradation by the 26 S proteasome. More recently, elucidating the role of mono-ubiquitylation as a sorting signal and regulator of endocytosis and endocytic trafficking led to the notion and subsequent demonstration that both mono- and polyubiquitylation regulate a much wider ar-

ray of cell processes, including cell cycle control, stress response, DNA repair, signaling, transcription, and gene silencing (reviewed in Ref. 1).

Ubiquitin conjugation is accomplished via a thioester cascade in which ubiquitin is first activated by a unique E1.<sup>1</sup> In a trans-esterification reaction ubiquitin is transferred from the ubiquitin activating enzyme to an E2 ubiquitin-conjugating enzyme, which may in turn transfer the ubiquitin molecule to an E3 ubiquitin-protein ligase. Specificity of the ubiquitylation system derives from the high fidelity protein-protein interactions between E3 and the substrate (reviewed in Ref. 2). Previous studies using a yeast temperature-sensitive mutant of the ubiquitin-activating enzyme (E1) indicated that the ubiquitin pathway is involved in both poly(A)<sup>+</sup> RNA export from the nucleus and in nuclear protein import (3). More recently, Tom1p and Rsp5p, two ubiquitin ligases (E3s) from the HECT family, have been shown to play a role in nuclear export of poly(A)<sup>+</sup> RNA in *Saccharomyces cerevisiae* (4–7). The ubiquitin ligase activity of both E3s is required for its function in mRNA export, suggesting that ubiquitylation of some mRNA nuclear transport factors contribute to the regulation of this transport pathway. The identification of the ubiquitin ligase substrates represents a major challenge to understand how this modification may modulate mRNA export.

mRNA export requires that newly synthesized precursor mRNAs undergo several processing steps, which include 5' capping, splicing, 3'-end cleavage, and polyadenylation. The different steps leading to mRNP formation are linked to each other and often mediated by interactions with the RNA polymerase II transcription machinery (8). All these functional couplings may be part of quality control mechanisms ensuring that only fully mature transcripts reach the cytoplasm (9). A considerable number of proteins have been implicated in the export of mRNA, including Mex67p (TAP in metazoan). Mex67p (TAP) acts as a transporter that interacts with export competent mRNPs and mediates its docking and translocation through the nuclear pore complexes. As Mex67p exhibits low affinity for RNA, its interaction with mRNAs is mediated by adaptor proteins like Yra1p, an essential hnRNP-like protein of the REF family (10–14). Recruitment of Yra1p and its partner Sub2p, an ATPase/RNA helicase essential for mRNA export, occurs co-transcriptionally and is splicing-independent (15–18). Recent findings established a more direct link between Yra1p, Sub2p, and the transcription machinery, since both

\* This work was supported in part by grants from the Association de Recherche contre le Cancer (ARC) and the Ministère délégué à la Recherche. 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.

§ Both authors contributed equally to this work.

¶ Supported by the CNRS.

‡ Supported by the Ministère délégué à la Recherche.

¶‡ To whom correspondence should be addressed: Inst. Jacques Monod, Unité Mixte de Recherche 7592, CNRS, Universités Paris VI and VII, 2 Place Jussieu, Tour 43, 75251 Paris Cedex 05, France. Tel./Fax: 33-1-44276956; E-mail: dargemont@ijm.jussieu.fr.

<sup>1</sup> The abbreviations used are: E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin-protein ligase; HA, hemagglutinin; GST, glutathione S-transferase; IPTG, isopropyl  $\beta$ -D-thiogalactopyranoside; GFP, green fluorescent protein; CTD, C-terminal domain; RNP, ribonucleoprotein; hnRNP, heterogenous RNP; mRNP, messenger RNP.

proteins are intimately associated to components of the THO complex, which has been implicated in transcription elongation, transcription-dependent recombination, and mRNA export (18–20). Genomic deletion of genes encoding THO complex components (*HPR1*, *THO2*, *MFT1*, *THP2*) is viable at 23 °C but leads to poly(A)<sup>+</sup> RNA export defect and cell death at 37 °C indicating that this complex is dispensable at 23 °C but essential at higher temperature (20). THO, Sub2p, and Yra1p are recruited coincidentally to an active gene during transcription elongation. Sub2p can be recruited to the nascent transcript by virtue of a direct interaction with Hpr1p and may in turn facilitate the binding of Yra1p to the mRNA (18). It was proposed that the binding of Mex67p to Yra1p at a later step displaces Sub2p from Yra1p prior mRNA export through the NPC (17, 21). In addition, it should be emphasized that the mRNA export function is not accomplished through a unique pathway and can involve additional adaptors (9).

To identify specific targets for the ubiquitin ligase Rsp5p whose ubiquitylation could regulate nuclear export of mRNA, we took advantage on two previously described properties of this enzyme. First, Rsp5p activity is dispensable for mRNA transport at 23 °C but essential at 30 or 37 °C. Second, besides its catalytic domain, the role of Rsp5p on nuclear transport is mediated by its second and third WW repeats (6) thought to interact with Rsp5p substrates through a PY motif (22–25). Here we show that the THO complex component Hpr1p is ubiquitylated and degraded both *in vitro* and *in vivo* by Rsp5p in conjunction with the E1 and Ubc4p as an E2.

#### EXPERIMENTAL PROCEDURES

**Yeast Strains and Growth**—The W303, YFS1675, DF5, *cim3.1*,  $\Delta$ erg,  $\Delta$ rsp5,  $\Delta$ tom1,  $\Delta$ ubc, and *rpb1-1* strains were described previously (5, 18, 26–31). *HPR1*, *HPR1* $\Delta$ 88, and *THP2* were genomically tagged with an HA epitope in a W303 background or *cim3.1* cells as described previously (32).  $\Delta$ rsp5 cells were grown in the presence of oleic acid as described (27, 6). CIM3 and *cim3.1* strains transformed with the His<sub>6</sub>-ubiquitin vector were grown on selective media supplemented with 0.1 mM of CuSO<sub>4</sub> (33). Cycloheximide, thiolutin, and MG132 were used at 100  $\mu$ g/ml, 10  $\mu$ g/ml, and 20  $\mu$ M final concentration, respectively.

**Protein Purification**—The DNA fragment encoding the 149 amino acids (281–429), corresponding to the WW2/WW3 repeats of Rsp5p, was cloned as a BamHI-Sall PCR fragment into pGEX4T-1 (Amersham Biosciences). BL21 strains expressing GST or GSTWW2/WW3 of Rsp5p were grown at 37 °C up to  $A_{600}$  = 0.6. Protein expression was induced overnight at 18 °C with 200  $\mu$ g/ml of IPTG. GST-Hpr1 fusion protein was expressed in *Escherichia coli* BL21 grown at 37 °C to an  $A_{600}$  of 0.6, submitted to cold and chemical shocks, and induced with 0.5 mM IPTG overnight at 23 °C. GST-Rsp5 (pGEX-6P2-Rsp5 expression plasmid was a generous gift from Dr. Linda Hicke), GST-CTD (pET21aGST-TEV-CTD was constructed by Dr. Nova Fong and generously provided by Dr. David Bentley) and His<sub>6</sub>-Ubc4 (from pET15b plasmid (34)) fusion proteins were expressed in *E. coli* BL21 grown at 37 °C to an  $A_{600}$  of 0.6 and induced with 1 mM IPTG overnight at 16 °C. Fusion proteins were purified on glutathione-Sepharose 4B beads (Amersham Biosciences) and nickel-agarose beads (Qiagen) according to the manufacturer's instructions. GST was cleaved off Rsp5p using the pre-scission protease (Amersham Biosciences) for 4 h at 4 °C in 50 mM Tris-HCl, pH 7.0, 150 mM NaCl, 1 mM dithiothreitol, 10% glycerol.

Purification of His<sub>6</sub>-tagged ubiquitin-Hpr1 conjugates was performed essentially as described (35) with minor modifications: 6 M guanidinium HCl buffer was supplemented with 0.1% Triton X-100 to obtain a yeast guanidinium buffer.

**GST Pull-downs**—100  $\mu$ g of GST or GST-WW2/WW3 proteins bound to 50  $\mu$ l of glutathione-Sepharose beads were incubated with cell extracts obtained from 10  $A_{600}$  of Bul1-HA, Hpr1-HA, Thp2-HA, or Mft1-GFP expressing cells (33).

**Antibodies**—Anti-Hpr1 and anti-Mex67p antibodies were generated by immunization of rabbits using recombinant GST-Hpr1 and His6-Mtr2/Mex67 complex (36), respectively (Charles River Laboratories). Anti-HA and anti-GFP monoclonal antibodies were purchased from Babco and Roche Applied Science, respectively. Anti-Mft1p and anti-GST antibodies were gift from Drs. T. Lithgow and T. Galli, respectively.

**Pulse-Chase Labeling and Immunoprecipitation**—Yeast cells were cultured in YNB medium with glucose to  $A_{600}$  of 0.8, concentrated to  $A_{600}$  of 5, then labeled at 23 °C for 25 min (pulse) with 150  $\mu$ Ci of Tran<sup>35</sup>S-label (PerkinElmer Life Sciences) per ml, prior to chase at 23 or 37 °C with 10 mM unlabeled methionine plus cysteine. Samples (0.3 ml) were withdrawn at different times of chase, total protein extracts were prepared by the NaOH-TCA lysis method, and proteins were processed for immunoprecipitation using anti-HA monoclonal antibodies as described previously (37). Proteins were separated by 8% SDS-PAGE and radioactivity was detected by fluorography.

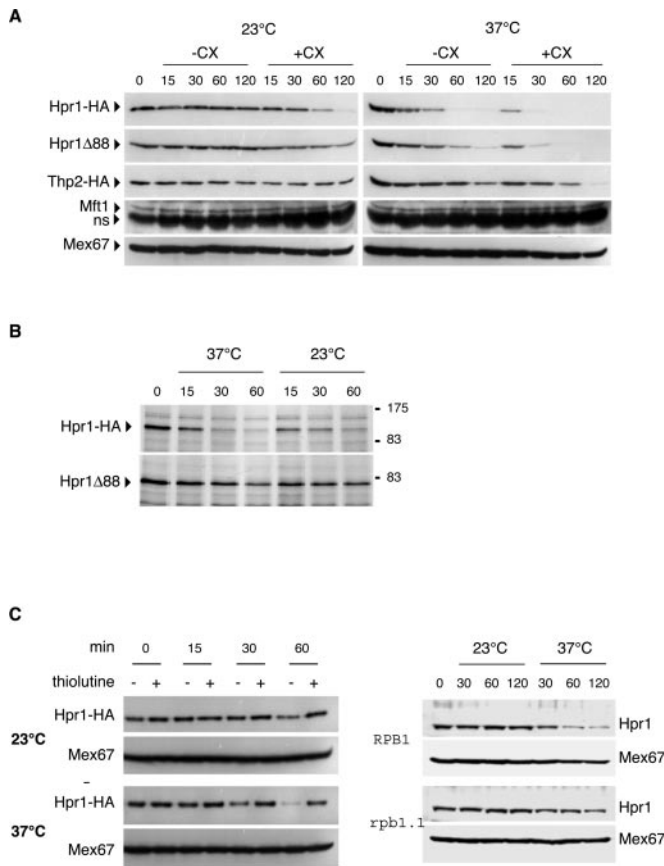
**Ubiquitylation Assay**—Standard ubiquitylation reactions contained 3  $\mu$ l of 5 $\times$  assay buffer (250 mM HEPES, pH 7.4, 25 mM MgOAc, 2.5 mM tris(2-carboxyethyl)phosphine, 500 mM NaCl, and 50% glycerol), 1  $\mu$ g of ubiquitin, 0.16  $\mu$ g of E1, 3.8  $\mu$ g of Ubc4 E2, 1.2  $\mu$ g of Rsp5 E3, 8 pmol of GST-tagged substrate, and 3.3 mM ATP when indicated. Reactions were allowed to proceed for 4 h at room temperature and stopped by boiling in 5  $\mu$ l of SDS-PAGE sample buffer containing 3 M urea.

#### RESULTS AND DISCUSSION

**Hpr1p Degradation Is Temperature-dependent**—It has been clearly shown that Rsp5p- and Tom1-dependent ubiquitylation, but also expression of the THO complex components, are dispensable for mRNA export at 23 °C but essential at 37 °C (6, 7, 20). To determine whether the THO complex, or at least one of its components, could be a substrate for the Rsp5p or Tom1p ubiquitin ligases resulting in the regulatory function of these enzymes in mRNA export, stability of the THO complex proteins was first analyzed in different conditions. The stability of THO components was measured at both 23 °C and 37 °C by cell treatment with the protein synthesis inhibitor cycloheximide (+CX) and analysis of expression by Western blotting using specific antibodies (Fig. 1A). Using this approach, genomically HA-tagged Hpr1p (Hpr1-HA) or untagged Hpr1p (data not shown) exhibited a half-life of about 30 min at 23 °C that was dramatically reduced to below 15 min at 37 °C (Fig. 1A). Hpr1p synthesis did not appear to fully compensate for this accelerated degradation, as indicated by the progressive decrease in the cellular content of Hpr1p upon a shift to 37 °C (–CX). Increased instability of Hpr1-HA at 37 °C was confirmed by pulse-chase experiment followed by immunoprecipitation of Hpr1-HA (Fig. 1B). In contrast to Hpr1p, the THO components Mft1p and genomically HA-tagged Thp2p, as well as the export receptor Mex67p, were rather stable at both 23 and 37 °C indicating that this highly active metabolism specifically concerns Hpr1p (Fig. 1A). Because of its low abundance (10-fold less than Mft1p), the stability of Thp2p was not analyzed in this study. Interestingly, deletion of the C-terminal 88 amino-acids that preserved the Hpr1p nuclear localization signal prevented degradation of Hpr1 $\Delta$ 88 at 23 °C and clearly slowed down its turnover at 37 °C (Fig. 1, A and B) indicating that the C-terminal domain of Hpr1p likely contains the target amino acid residues for accelerated degradation. Deletion of this region does not strongly affect viability at 37 °C suggesting that this truncation does not prevent formation of the THO complex.

**Degradation of Hpr1p Depends on Active RNA Polymerase II-mediated Transcription**—Proteins of the THO complex co-purify with the DEAD-box RNA helicase Sub2p and the mRNA export adaptor Yra1p in a complex called TREX, proposed to couple transcription and nuclear export (20). Studies on the THO components of TREX indicate that the recruitment of Hpr1p occurs during transcription elongation (18, 20, 38). We thus analyzed the requirement of the transcription process in the ubiquitin-proteasome-mediated degradation of Hpr1p. For this purpose, cells were treated with the transcription inhibitor thiolutin for 15 min prior to shifting the cells to 37 °C. As shown on Fig. 1C, thiolutin led to a clear stabilization of Hpr1p at 37 °C. To confirm this result, Hpr1p expression was also analyzed in cells containing a thermosensitive mutation for Rpb1, the large RNA polymerase II subunit. We found that

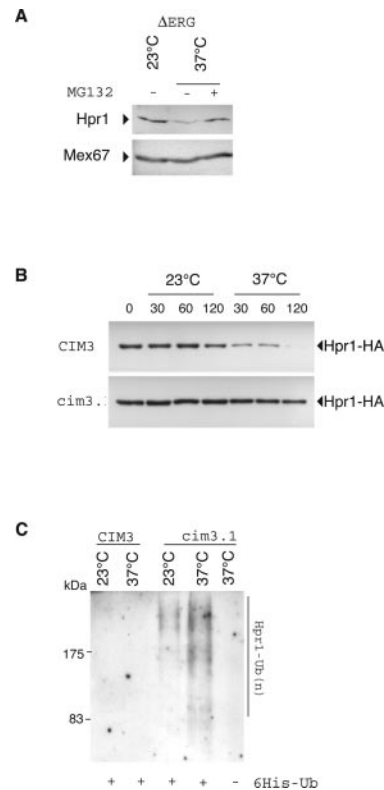




**FIG. 1. Degradation of Hpr1p is temperature- and transcription-dependent.** A, the stability of full-length or truncated Hpr1-HA, Thp2-HA, Mft1p, and Mex67p was analyzed in cells treated in the presence or absence of cycloheximide (CX) for the indicated times (minutes) at 23 °C or 37 °C. Total cell extracts were examined by Western blot with the corresponding antibodies. B, cells expressing full-length or truncated Hpr1-HA were labeled with Tran<sup>35</sup>S-label for 25 min and chased for the indicated period of time in the presence of 10 mM unlabeled methionine plus cysteine. Hpr1-HA proteins were immunoprecipitated from total cell extracts and analyzed by SDS-PAGE and fluorography. C, cells expressing an HA-tagged version of Hpr1p were treated (+) or not (–) with 10 μg/ml of thiolutin for the indicated periods of time (minutes (min)) and temperatures (°C). Alternatively, Hpr1p expression was determined in wild-type *RPB1* or the *rpb1-1* temperature-sensitive mutant. Cell extracts were analyzed as described for A.

degradation of Hpr1p was mostly prevented in these cells at 37 °C (which also corresponds to the restrictive temperature), whereas the level of Mex67p remained stable (Fig. 1C). These results illustrate that on-going transcription is required for Hpr1p turnover.

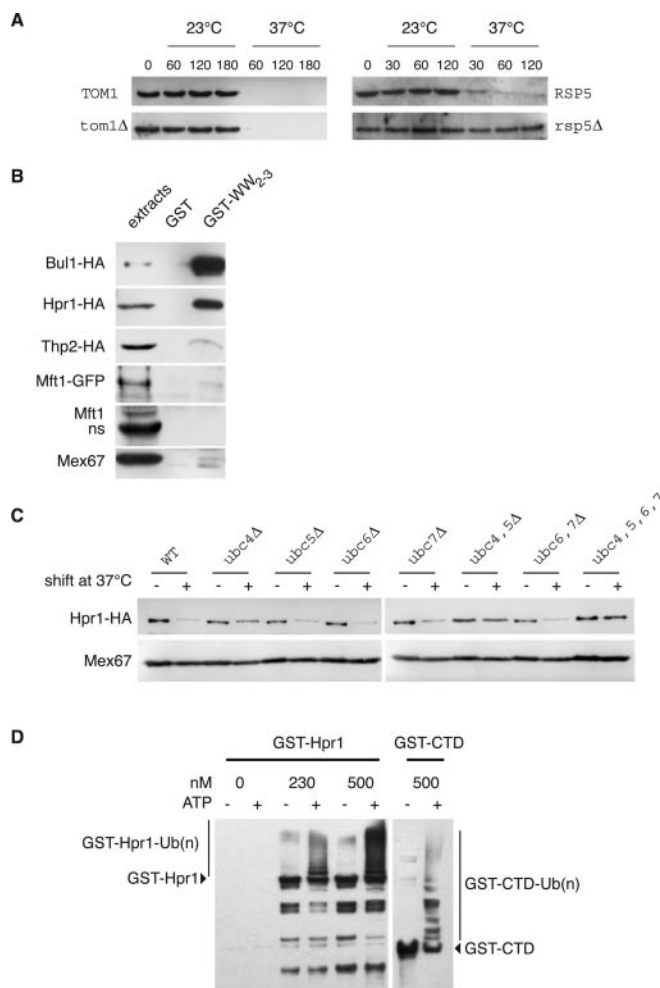
**Ubiquitin- and Proteasome-dependent Degradation of the THO Complex Factor Hpr1p**—To determine whether degradation of Hpr1p was dependent on the proteasome activity, the sensitivity of Hpr1p expression to proteasome inhibitor was analyzed in  $\Delta erg$  cells, which enable the uptake of peptide inhibitors (29). Results shown in Fig. 2A clearly indicated that degradation of Hpr1p at 37 °C was prevented in cells treated with a 20 μM concentration of the proteasome inhibitor MG132. Consistent with these data, Hpr1p levels were stabilized at 37 °C in *cim3.1* thermosensitive mutants, impaired in proteasome activity (28), compared with the corresponding wild-type *CIM3* strain (Fig. 2B). Since recognition and degradation by the 26 S proteasome requires polyubiquitination of the substrate, modification of Hpr1p was analyzed in wild-type and *cim3.1* cells overexpressing His-tagged versions of ubiquitin from a copper-inducible vector. Cells were maintained at 23 °C or shifted to 37 °C for 4 h. His<sub>6</sub>-ubiquitin-conjugated proteins



**FIG. 2. Ubiquitin- and proteasome-dependent degradation of Hpr1p.** A, Hpr1p expression was analyzed in  $\Delta erg$  cells maintained at 23 °C or shifted to 37 °C during 1 h in the absence or presence of 20 μM MG132. B, Hpr1-HA expression was similarly analyzed in wild-type and *cim3.1* cells maintained at 23 °C or shifted to 37 °C during the indicated periods of time (minutes). C, nickel columns were used to purify His<sub>6</sub>-ubiquitin conjugated forms of Hpr1p from *cim3.1* cells maintained at 23 °C or shifted to 37 °C during 4 h. Cell extracts or purified material was examined by Western blot with an anti-Hpr1p antibody.

from the cell lysates were purified on nickel columns and analyzed by Western blotting using an anti-Hpr1p antibody. With this approach, polyubiquitin-conjugated species of Hpr1p could be detected in extracts of *cim3.1* cells expressing His<sub>6</sub>-tagged ubiquitin and grown at the restrictive temperature. In contrast, wild-type cells and mutant cells grown at the permissive temperature or transformed with a control vector presented a weak or no detection of ubiquitylated Hpr1p (Fig. 2C). Altogether, these results clearly indicate that Hpr1p is polyubiquitylated and actively metabolized by the proteasome-mediated degradation pathway.

**Hpr1p Is a Substrate for the Rsp5p Ubiquitin Ligase**—The catalytic activity of the ubiquitin ligases Rsp5p and Tom1p has been shown to regulate efficiency of mRNA nuclear export (5–7). To determine whether one of these ligases was responsible for Hpr1p ubiquitylation, Hpr1p degradation was analyzed in *rsp5Δ* or *tom1Δ* strains. Although disruption of the *RSP5* gene is lethal, cell viability at 23 °C, but not at 37 °C, can be rescued by adding oleic acid to the growth medium (27). Indeed the processing-mediated activation of the transcription factor Spt23p by Rsp5p is required for the synthesis of Ole1p, an endoplasmic reticulum-bound enzyme essential for lipid and membrane synthesis which catalyzes the desaturation of C16 and C18 fatty acids and formation of palmitoleic and oleic acids. Disruption of *TOM1* or *RSP5* did not affect the steady-state expression of Hpr1p at 23 °C. In contrast, Hpr1p degradation was completely abolished at 37 °C in a *rsp5Δ* strain and not in a *tom1Δ* strain (Fig. 3A), suggesting that ubiquitylation of Hpr1p is likely to be mediated by the Rsp5p ubiquitin ligase.



**FIG. 3. Ubiquitylation of Hpr1p is mediated by the ubiquitin-protein ligase Rsp5p and the ubiquitin conjugation enzyme Ubc4p.** *A*,  $\Delta$ *rsp5* and  $\Delta$ *tom1* as well as the corresponding wild-type cells were grown at 23 °C or shifted to 37 °C during the indicated periods of time (minutes). *B*, GST or GST-WW2/WW3 Rsp5 recombinant proteins on glutathione-Sepharose beads were incubated with extracts from cells expressing HA-tagged versions of Bul1, Hpr1p, or Thp2p or GFP-tagged Mft1p. Untagged Mft1p and Mex67p were analyzed from cells expressing Hpr1-HA. Beads were washed, and bound material was analyzed by Western blotting. 0.5% of the input was loaded in lane 1 (extracts). *C*, indicated cell strains were grown at 23 °C or shifted to 37 °C. *D*, ubiquitylation of indicated amount of GST-Hpr1 or GST-CTD was realized in the presence of recombinant E1, Ubc4, and Rsp5. ATP was either omitted or added last to minimize auto-catalytic ubiquitylation reactions by the ubiquitylation enzymes. Cell extracts or *in vitro* reactions were analyzed by Western-blot with indicated antibodies.

To further investigate whether Hpr1p specifically interacts with Rsp5p, we generated a GST fusion protein expressing the WW2/WW3 domains (amino acids 281–429) of this E3 ligase, a region previously implicated in the nuclear export of poly (A)<sup>+</sup> RNA (6). A lysate from cells expressing an HA-tagged version of the Rsp5p partner Bul1p was used to test the functionality of recombinant GST-WW2/WW3 in a GST pull-down assay. Using the same binding conditions we observed a specific and strong interaction of Bul1p and Hpr1p to the GST-WW2/WW3 column but not to the GST control (Fig. 3B). A similar binding of Hpr1p to the WW2/WW3 domains has been observed in *mft1Δ* cells indicating that interaction between Rsp5p and Hpr1p can occur in the absence of a functional THO complex (data not shown). In contrast Thp2p and Mft1p poorly bound to the GST-WW2/WW3 column and Mex67p displayed no significant interaction with this fusion protein (Fig. 3B). We conclude that the WW2/WW3 domains of Rsp5p are responsible for Hpr1p recognition.

Covalent attachment of ubiquitin molecules is mediated through a thiol ester cascade of reactions catalyzed by a unique ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin-protein ligase (E3). Both ubiquitin-conjugating enzymes Ubc4p and Ubc5p have been described as partners for the Rsp5p ligase (39). To test whether one of these enzymes is required for the ubiquitylation and subsequent degradation of Hpr1p, Hpr1p expression was analyzed at 23 °C or after a 1-h shift at 37 °C in *ubc4Δ*, *ubc5Δ*, *ubc4Δ ubc5Δ* mutant cells. As a control, we also used cells disrupted for *UBC6* and/or *UBC7* genes encoding for ubiquitin-conjugating enzyme involved in particular in the degradation of membrane proteins (31). As shown in Fig. 3C, temperature-induced Hpr1p degradation was not affected in *ubc5Δ*, *ubc6Δ*, *ubc7Δ* cells but was prevented in *ubc4Δ* cells with a more pronounced effect in the absence of both Ubc4p and Ubc5p enzymes. Although Ubc4p and Ubc5p have been shown to be highly similar and functionally redundant, *UBC4* is mainly transcribed during the exponential phase of growth and stimulated by heat shock, whereas transcription of *UBC5* occurs during the stationary phase (30). This differential pattern of expression could explain the predominant but not exclusive role of Ubc4p in the ubiquitylation of Hpr1p at 37 °C. Altogether these results show that Ubc4p, and probably Ubc5p, and Rsp5p are required for the ubiquitylation of Hpr1p *in vivo*.

*E1, Ubc4p, and Rsp5p Are Necessary and Sufficient to Ubiquitylate Hpr1p in Vitro*—To test whether E1, Ubc4p, and Rsp5p are not only required but also sufficient to promote polyubiquitylation of Hpr1p, *in vitro* ubiquitin conjugation reactions with recombinant GST-Hpr1 were carried out in the presence of recombinant purified enzymes. GST fused to the C-terminal domain (CTD) of RNA polymerase II large subunit was used as a control (40). As shown in Fig. 3D, a marked increase in the amount of high molecular weight conjugates and a corresponding decrease in the level of unmodified GST-Hpr1 and GST-CTD was observed in reactions performed in the presence of ATP. No modification could be detected when the assay was performed in the absence of one of the enzymes or in the presence of Ubc1p as an alternative E2 (not shown). In addition, modification of GST-Hpr1 appeared dependent on the concentration of the substrate and reached a plateau with 500 nM GST-Hpr1 (Fig. 3D and data not shown). Although we cannot formally exclude that binding of Rsp5p to Hpr1p can be regulated by an additional partner *in vivo*, results presented here clearly demonstrate that interaction between this enzyme-substrate complex does not require any adaptor *in vitro* and might involve the recognition of the LPxY motif of Hpr1p by the WW2/WW3 repeats of Rsp5p (22–25). The important proportion of the high molecular weight forms of Hpr1p observed in the *in vitro* Hpr1p ubiquitin conjugating assay as compared with the positive control CTD, indicate that Hpr1p is a privileged target of Rsp5p. This finding is supported by data from Kus and Edwards,<sup>2</sup> who found Hpr1p as one of the top10 substrates of Rsp5p using a high throughput screen for the identification of substrates for Rsp5p.

Data shown here clearly indicate that the stability of the mRNA nuclear export factor Hpr1p is controlled by the ubiquitin/proteasome pathway, whereas the other members of the THO complex such as Mft1p or Thp2p are much more stable. This suggests that Hpr1p represents a master piece whose stability could control the integrity and activity of the whole THO complex. In agreement with this model, we found that instability of Hpr1p at 37 °C promotes increased dissociation rate of the THO complex (data not shown). Because the turn-

<sup>2</sup> B. Kus and A. M. Edwards, personal communication.

over of Hpr1p depends on an active on-going transcription, we would like to propose that dissociation of the THO complex may be coupled to the transcription process and possibly involved in mature mRNP release. Defects in Rsp5p ligase activity or in Hpr1p expression would thus have direct consequences on mRNA export as it has been previously observed (6, 7, 20). Evidence has been accumulating over the last few years suggesting the existence of multiple mRNA export pathways defined by the association of variable combinations of mRNA-binding proteins, adaptors, and export receptors to nascent transcripts. Identification of the Rsp5p as a regulator of mRNA export and association of Rsp5p with the transcription machinery lead us to predict a key role of this enzyme in the regulation and maybe the coordination of the early mRNP assembly and remodeling events responsible for the formation of export-competent mRNP.

**Acknowledgments**—We thank S. Jentsch, C. Guthrie, D. Grimal-Urban, T. Lithgow, M. Christman, and P. Legrain for plasmids, strains, and antibodies. We also thank F. Stutz, R. Haguenaer-Tsapis, and D. Libri for reagents, helpful discussions, and critical reading of the manuscript.

## REFERENCES

- Glickman, M. H., and Ciechanover, A. (2002) *Physiol. Rev.* **82**, 373–428
- Pickart, C. M. (2001) *Mol. Cell* **8**, 499–504
- Azad, A. K., Tani, T., Shiki, N., Tsuneyoshi, S., Urushiyama, S., and Ohshima, Y. (1997) *Mol. Biol. Cell* **8**, 825–841
- Utsugi, T., Hirata, A., Sekiguchi, Y., Sasaki, T., Toh-e, A., and Kikuchi, Y. (1999) *Gene (Amst.)* **234**, 285–295
- Duncan, K., Umen, J. G., and Guthrie, C. (2000) *Curr. Biol.* **10**, 687–696
- Rodriguez, M. S., Gwizdek, C., Haguenaer-Tsapis, R., and Dargemont, C. (2003) *Traffic* **4**, 566–575
- Neumann, S., Petfalski, E., Brugger, B., Grosshans, H., Wieland, F., Tollervey, D., and Hurt, E. (2003) *EMBO Rep.* **4**, 1156–1162
- Maniatis, T., and Reed, R. (2002) *Nature* **416**, 499–506
- Rodriguez, M. S., Dargemont, C., and Stutz, F. (2004) *Biol. Cell* **96**, 639–655
- Katahira, J., Strasser, K., Podtelejnikov, A., Mann, M., Jung, J. U., and Hurt, E. (1999) *EMBO J.* **18**, 2593–2609
- Santos-Rosa, H., Moreno, H., Simos, G., Segref, A., Fahrenkrog, B., Pante, N., and Hurt, E. (1998) *Mol. Cell. Biol.* **18**, 6826–6838
- Strasser, K., and Hurt, E. (2000) *EMBO J.* **19**, 410–420
- Stutz, G., Martini, A. C., Ruiz, R. D., Fiol De Cuneo, M., Munoz, L., and Lacuara, J. L. (2000) *Arch. Androl.* **44**, 117–128
- Zenkhusen, D., Vinciguerra, P., Strahm, Y and Stutz, F. (2001) *Mol. Cell. Biol.* **21**, 4219–4232
- Lei, E. P., Krebber, H., and Silver, P. A. (2001) *Genes Dev.* **15**, 1771–1782
- Jensen, T. H., Boulay, J., Rosbash, M., and Libri, D. (2001) *Curr. Biol.* **11**, 1711–1715
- Strasser, K., and Hurt, E. (2001) *Nature* **413**, 648–652
- Zenkhusen, D., Vinciguerra, P., Wyss, J. C., and Stutz, F. (2002) *Mol. Cell. Biol.* **22**, 8241–8253
- Chavez, S., Beilharz, T., Rondon, A. G., Erdjument-Bromage, H., Tempst, P., Svejstrup, J. Q., Lithgow, T., and Aguilera, A. (2000) *EMBO J.* **19**, 5824–5834
- Strasser, K., Masuda, S., Mason, P., Pfannstiel, J., Oppizzi, M., Rodriguez-Navarro, S., Rondon, A. G., Aguilera, A., Struhl, K., Reed, R., and Hurt, E. (2002) *Nature* **417**, 304–308
- Reed, R., and Hurt, E. (2002) *Cell* **108**, 523–531
- Bork, P., and Sudol, M. (1994) *Trends Biochem. Sci.* **19**, 531–533
- Chen, H. I., and Sudol, M. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 7819–7823
- Macias, M. J., Hyvonen, M., Baraldi, E., Schultz, J., Sudol, M., Saraste, M., and Oschkinat, H. (1996) *Nature* **382**, 646–649
- Shcherbik, N., Kee, Y., Lyon, N., Huibregtse, J. M., and Haines, D. S. (2004) *J. Biol. Chem.* **279**, 53892–53898
- Mayer, T. U., Braun, T., and Jentsch, S. (1998) *EMBO J.* **17**, 3251–3257
- Hoppe, T., Matuschewski, K., Rape, M., Schlenker, S., Ulrich, H. D., and Jentsch, S. (2000) *Cell* **102**, 577–586
- Ghislain, M., Udvardy, A., and Mann, C. (1993) *Nature* **366**, 358–362
- Lee, D. H., and Goldberg, A. L. (1998) *Trends Cell Biol.* **8**, 397–403
- Seufert, W., and Jentsch, S. (1990) *EMBO J.* **9**, 543–550
- Chen, P., Johnson, P., Sommer, T., Jentsch, S., and Hochstrasser, M. (1993) *Cell* **74**, 357–369
- Longtine, M. S., McKenzie, A., III, Demarini, D. J., Shah, N. G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J. R. (1998) *Yeast* **14**, 953–961
- Cohen, M., Stutz, F., Belgareh, N., Haguenaer-Tsapis, R., and Dargemont, C. (2003) *Nat. Cell Biol.* **5**, 661–667
- Kus, B. M., Caldon, C. E., Andorn-Broza, R., and Edwards, A. M. (2004) *Proteins* **54**, 455–467
- Rodriguez, M. S., Desterro, J. M., Lain, S., Midgley, C. A., Lane, D. P., and Hay, R. T. (1999) *EMBO J.* **18**, 6455–6461
- Strasser, K., Bassler, J., and Hurt, E. (2000) *J. Cell Biol.* **150**, 695–706
- Moreau, V., Galan, J. M., Devilliers, G., Haguenaer-Tsapis, R., and Winsor, B. (1997) *Mol. Biol. Cell* **8**, 1361–1375
- Kim, M., Ahn, S. H., Krogan, N. J., Greenblatt, J. F., and Buratowski, S. (2004) *EMBO J.* **23**, 354–364
- Nuber, U., and Scheffner, M. (1999) *J. Biol. Chem.* **274**, 7576–7582
- Huibregtse, J. M., Yang, J. C., and Beaudenon, S. L. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 3656–3661

## **The mRNA Nuclear Export Factor Hpr1 Is Regulated by Rsp5-mediated Ubiquitylation**

Carole Gwizdek, Maria Hobeika, Bart Kus, Batool Ossareh-Nazari, Catherine Dargemont and Manuel S. Rodriguez

*J. Biol. Chem.* 2005, 280:13401-13405.

doi: 10.1074/jbc.C500040200 originally published online February 15, 2005

---

Access the most updated version of this article at doi: [10.1074/jbc.C500040200](https://doi.org/10.1074/jbc.C500040200)

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 40 references, 18 of which can be accessed free at <http://www.jbc.org/content/280/14/13401.full.html#ref-list-1>