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Regulation of TORC1 by Ubiquitin through Non-covalent Binding

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Abstract

Ubiquitin (Ub) regulates numerous cellular processes through covalent attachment to other proteins in the forms of poly- and mono-ubiquitination. A recent study in yeast shows that ubiquitin controls TORC1 through a noncovalent binding with Kog1, a regulatory subunit of TORC1. The binding stabilizes Kog1 and prevents its degradation under stress conditions. This finding unveils a novel role of Ub in TORC1 function and implicates a unique mechanism that attributes the action of Ub in cell signaling.

Keywords

ubiquitin; Kog1; TOR; rapamycin; WD40 repeats

Ubiquitin (Ub) is a small protein of 76 amino acids that is ubiquitously expressed in all eukaryotic cells. It functions as a unique regulatory component of various cellular processes through covalent attachment to many proteins (Heride et al. 2014; Komander & Rape. 2012). This posttranslational modification occurs in the forms of polyubiquitination and monoubiquitination. In the former case, the glycine at the C-terminus of ubiquitin is linked to a lysine in a target protein and additional Ubs then attach to lysine 48 in the proceeding ubiquitin to form a polyubiquitin chain. This form of ubiquitination is generally used in targeting proteins for proteasomal degradation (Hochstrasser. 1996). Monoubiquitination involves a single ubiquitin that is attached to a target protein. This form of ubiquitination functions in a way similar to phosphorylation or acetylation, resulting in changes in protein conformation, interaction and intracellular localization (Hicke & Dunn. 2003; Mukhopadhyay & Riezman. 2007). In addition, many proteins contain ubiquitin binding domains (UBD) that are able to interact with ubiquitin noncovalently. This type of proteins often functions as adaptors of ubiquitin E3 ligases or ubiquitin shuttle factors that are responsible for binding and targeting ubiquitinated proteins to proteasome. Many structurally distinct UBDs have been identified, including ubiquitin-association domain, ubiquitin interacting domain and ubiquitin binding zinc fingers (Grabbe & Dikic. 2009; Hoeller et al. 2007). A previous study revealed that WD40 repeats, which form a circular β -propeller structure found in a wide range of proteins, could also serve as a UBD (Pashkova et al. 2010). Ub was shown to bind the WD40 repeat domain in Cdc4, an F-box protein for

the SCF ubiquitin E3 ligase, and facilitate its autoubiquitination and degradation. In addition, Ub was found to interact with other WD40 repeat containing proteins that were not part of ubiquitin E3 ligases or involved in ubiquitination. Among those was Kog1, a key regulatory component of the Target of Rapamycin Complex 1 (TORC1) in yeast (Pashkova et al. 2010). The biological significance of the binding remained unclear. However, a recent study by Hu et al unveils a role of this ubiquitin binding in regulation of TORC1. Surprisingly, the action of Ub in the regulation does not involve covalent attachment, indicating that Ub is able to regulate TORC1 independent of ubiquitination (Hu et al. 2015).

TORC1 is a central regulator of cell growth and metabolism in eukaryotic cells. It elicits its function through integration and coordination of signals of various origins, including nutrient, growth factors, energy and stresses (Ho & Gasch. 2015; Zoncu et al. 2011). In yeast *Saccharomyces cerevisiae* TORC1 is composed of several components, including the Tor proteins, Kog1, Lst8 and Tco89, of which the Tor proteins, Tor1 and Tor2, serve alternatively as the catalytic subunit and Kog1 functions as the regulatory subunit to define the substrate specificity of the complex. The function of TORC1 is sensitive to macrolide drug rapamycin. The drug, in complex with a small cytosolic protein FKBP12, binds to a region in the Tor proteins termed as the FKBP12-rapamycin binding (FRB) domain and disrupts the function of the Tor proteins (Wullschleger et al. 2006). In an attempt to define the role of the FRB domain in the normal function of the Tor proteins, Hu et al created a series of mutations in the domain of Tor2 and identified a mutation, Tor2^{W2041R}, that rendered yeast cells temperature sensitive and rapamycin resistant. Analysis of the cells bearing the *tor2* mutant revealed that the mutant protein was defective for binding with Kog1. Surprisingly, it was found that at the nonpermissive temperature, Kog1, rather than the mutant Tor2 protein, was rapidly degraded. This observation indicates that Kog1 is a labile protein that requires association with the Tor proteins for stabilization. A genetic screen identified Ub as a suppressor that when overexpressed was able to stabilize Kog1 and suppress the growth defect of the mutant cells at the nonpermissive temperature. The latter finding uncovered a novel role for Ub in protein stabilization (Hu et al. 2015).

The protective effect of Ub on Kog1 does not appear to involve ubiquitination, as there is no evidence showing that Ub is covalently attached to Kog1 or other components of TORC1 (Hu et al. 2015). The major lysine residues in Ub involved in ubiquitination are not essential for the suppressive activity of Ub. In contrast, Ub was found to noncovalently associate with Kog1 and prevent its degradation in cells under heat stress. An Ub mutant that was unable to interact with Kog1 was ineffective in suppressing the growth defect of the mutant cells. These observations suggest that the association with Ub stabilizes Kog1 under heat stress through noncovalent binding.

How does Ub prevent Kog1 from degradation? In the case of F-box protein Cdc4, it has been suggested that E2-linked Ub binds to the WD40 repeat domain of the protein and transfers the activated Ub to a lysine acceptor within the domain for ubiquitination. This unique E3 ligase independent ubiquitination is believed to promote subsequent polyubiquitination and degradation of Cdc4 (Pashkova et al. 2010). It is thus possible that the binding of free Ub to the WD40 repeat domain of Kog1 blocks the access of E2-linked Ub, hence preventing Kog1 from ubiquitination and degradation (Fig. 1). Alternatively, the binding of Ub may

block an E3 ligase from accessing Kog1 that is marked for ubiquitination by heat stress induced phosphorylation. In this regard, it would be informative to examine the stability of a mutant Kog1 protein that is defective for binding with Ub, which can be achieved by altering the residues in the WD40 repeat domain involved in Ub binding, as it has been demonstrated in the case of Cdc4 (Pashkova et al. 2010). It is worth noting that the WD40 repeat domain of Kog1 is involved in association with the Tor proteins (Adami et al. 2007). The binding of Ub to the same region of Kog1 is thus expected to affect its association with the Tor proteins. A previous study has shown that under heat stress, TORC1 is transiently dislodged from membrane and becomes cytosolic (Yan et al. 2012). The binding of Ub to Kog1 under the stress condition may stabilize the protein and prevent TORC1 from disassembly.

In addition to Kog1, several other proteins containing WD40 repeat domain that are not part of E3 ligases are found to interact with Ub (Pashkova et al. 2010). It can be postulated that binding of Ub may affect the function and/or stability of these proteins, as it does to Kog1. Further study of this noncovalent binding of Ub to Kog1 and other proteins containing WD40 repeat domain will provide insights regarding the role and significance of this novel role of Ub in cell signaling.

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Figure 1. A working model for the role of Ub in Kog1 regulation

Ub binds to the WD40 repeat domain of Kog1 and prevents the access by E2-linked Ub in an E3 ligase complex (**A**). In the absence of Ub binding Kog1 is ubiquitinated and targeted for degradation under stress condition (**B**).