Two Ubiquitin-conjugating Enzyme Variants Regulate Different Cellular Stress Responses

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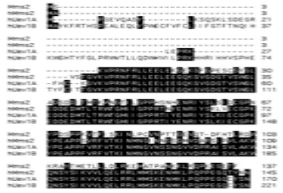
Abstract

The ubiquitin conjugating (Ubc) enzyme, Ubc13, in concert with the ubiquitin conjugating enzyme variant (Uev) Mms2 is essential for DNA error-free post-replication repair (PRR) in budding yeast by promoting noncanonical lysine-63 ubiquitin polymerization of PCNA. Mammalian cells contain two Mms2 homologs, Uev1A and Mms2, with greater than 90% amino acid sequence identity to the core region and both are able to interact with Ubc13. Furthermore, both Mms2 and Uev1A are capable of substituting for the yeast Mms2 mutant, suggesting the presence of Ubc13-Uev mediated error-free PRR in mammalian cells. Alternatively, in mammalian cells Ubc13 is reported to interact with a Uev (Uev1A or Mms2) to regulate the NF-κB signal transduction pathway by poly-ubiquitinating NEMO/IKKgamma. Based on the above observations, we hypothesized that the Ubc13 activity to ubiquitylate different substrates is dependent on its physical interaction with different Uevs. Using RNA interference (RNAi), myc-tagged constructs and immunocytochemistry, we demonstrated that suppression of either Ubc13 or Uev1A resulted in loss of NEMO polyubiquitination and NF-κB translocation to the nucleus while suppression of Mms2 has not effect. Alternatively, repression of Ubc13 or Mms2, but not Uev1A, resulted in increased numbers of Rad51 positive nuclei, indicating the increase in spontaneous DNA damage. Additionally, Ubc13 co-localizes with myc-tagged Mms2 in response to DNA damage, while myc-tagged Uev1A was not observed to form nuclear foci. Furthermore, removal of the N-terminal unique region of Uev1A resulted in its co-localization with Ubc13 nuclear foci after DNA damage, reminiscent of the Ubc13-Mms2 nuclear foci. We conclude that Mms2 and Uev1A serve as regulatory subunits of the Ubc13-Uev complex and direct it to two distinct pathways, namely the DNA repair pathway to deal with genotoxic stress and the NF-κB pathway in response to nongenotoxic stresses such as bacterial or viral infection.



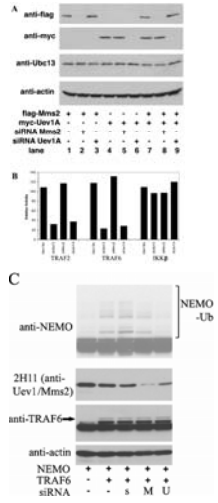
Amino acid sequence comparison of yeast Mms2 and its human homologues.

The alignment demonstrates a high amino acid similarity in the core regions of Uev1A and Mms2, with significant diversity in the N-terminal.

Ubc13

+ Uev1A → NF-κB activity

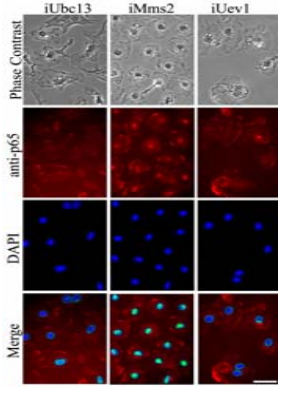
+ Mms2 → DNA repair activity



Ablation of Uev1A suppresses TRAF-induced NF-κB activity and NEMO polyubiquitinylation in 293T cells.

A. Western blot analysis confirms RNAi specificity.
B. Traf-2 and Traf-6 activity is dependent on Ubc13 and Uev1, not Mms2.
C. NEMO ubiquitinylation is dependent upon Uev1A

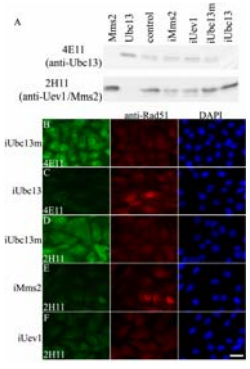
This demonstrates that the Ubc13-Uev1A complex is required for TRAF activation of NF-κB, whereas Mms2 is not required.



LPS-induced p65 translocation to the nucleus is dependent upon Ubc13 and Uev1A, not Mms2.

Phase contrast, p65 ICC, and DAPI staining of macrophage treated for 1.5hr with 1ug/ml LPS following RNAi suppression of the indicated target proteins. The merged images have been adjusted to accentuate differential co-localization.

This demonstrates a physiological role of the Ubc13-Uev1A complex in NF-κb activation, independently of Mms2.

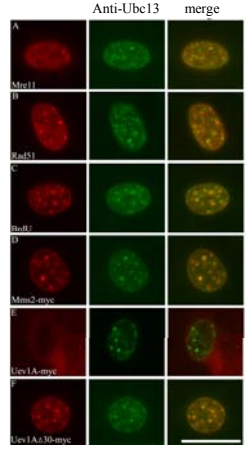


siRNA	Total cells counted	% of Rad51 positive nuclei	Standard deviation	p* (with respect to control)
UBC13m	1010	8	±1.3	- -
UBC13	991	33	±2.5	< 0.001
MMS2	970	32	±2.5	< 0.001
UEV1A	1011	8	±1.7	1.0

Spontaneous Rad51 nuclear foci formation after RNAi repression of Ubc13 and or Mms2.

A. Werstern blot analysis confirms RNAi specificity.
B-F. Demonstration of spontaneous Rad51 foci formation following RNAi suppression of the indicated target proteins.
G. Inventory of Rad51 foci contaning cells following RNAi treatment

This demonstrates a function of the Ubc13-Mms2 complex in genome stability independently of Uev1A as is expected from previous yeast research.



Camptothecin-induced nuclear foci formation of Ubc13 and Mms2-myc.

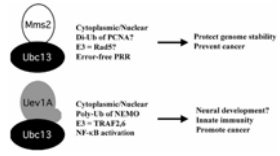
Mild detergent pre-extraction of 3T3 cells following 4 hours of exposure to the topoisomerase inhibiter camptotheicin results in co-localization of Ubc13 and Mms2-myc in the nucleus. Uev1A-myc was not observed to form foci until the N-terminal was removed.

This demonstrates an involvement of the Ubc13-mms2 complex in response to induced DNA damage independently of Uev1A.

Conclusions

1. The Ubc13-Uev1A (but not Mms2) complex is involved in ubiquitinylation of NEMO and activation of the NF-κB pathway.
2. The Ubc13-Mms2 (but not Uev1A) complex is involved in spontaneous and induced DNA damage activity, suggesting the existence in a mammalian DNA post-replication repair activity.
3. Mms2 and Uev1A are not transposable.
4. The N-terminus likely specifies activity of the Uev.

This research describes two alternative activities of Ubc13 in mammalian cells which is dependent upon the particular Uev involved. Specificity may be dependent upon the N-terminal extension of the Uev.



A working model of Ubc13-Uev in mammalian cells.

Acknowledgments

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