Mammalian ubiquitin conjugating enzyme complex Ubc13-Mms2 is involved in error-free DNA postreplication repair.

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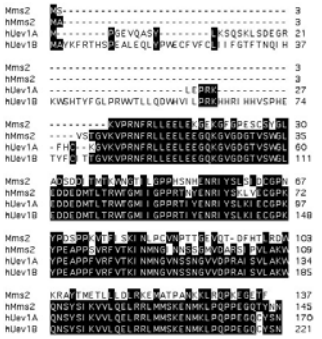
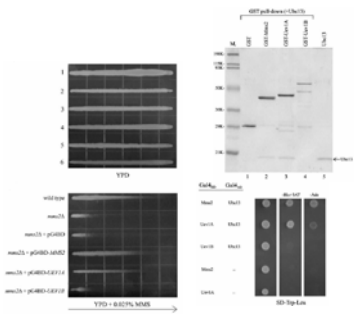
Abstract

Error-free DNA post replication repair (PRR) in budding yeast is dependent upon Lys63-mediated polyubiquitination chain formation catalyzed by the Ubc13-Mms2 complex. In this model organism, inactivation of *UBC13* or *MMS2* favors error-prone translesion DNA synthesis (TLS), leading to genome instability. These genes appear to be conserved throughout higher eukaryotes; however the mammalian genome contains an additional homologue of *MMS2*, *UEV1*, suggesting an increase in genome complexity. Human Mms2 (145 aa) and two Uev1 splice variants, Uev1A (170aa) and Uev1B (221aa), share >90% sequence identity in their core domains and constitute a novel family known as ubiquitin-conjugating enzyme variants (Uevs). The earlier observation that *UEV1* is highly expressed in multiple tumor cell lines led us to hypothesize that Uev1 has the potential to sequester Ubc13 into cellular activities other than PRR and in so doing has the potential to promote mutagenic TLS. Initial observations in yeast indicate that hMms2 and hUev1A (but not hUev1B) can complement the *mms2* defect in PRR, which leads us to investigate the roles of Mms2 and Uev1 in mammalian cells. By examining native Ubc13 and myc-tagged Uevs, we identified a nuclear Ubc13-Mms2 complex distinct from Uev1A or Uev1B. Ubc13 and Mms2-myc were found to be localized in the nucleus coinciding with both replicative DNA synthesis during log-phase growth, and at sites of unscheduled DNA synthesis following treatment with camptothecin, a chemotherapeutic agent producing DNA strand breaks. Experimental reduction of either Ubc13 or Mms2 using interference RNA (iRNA) technology resulted in an increase in Rad51 positive nuclei, indicative of spontaneous DNA double-strand breaks. Furthermore, simultaneous reduction of Rev3 and either Ubc13 or Mms2 by iRNA resulted in an increased susceptibility to UV, strongly suggesting the involvement of Ubc13-Mms2 in error-free PRR in mammalian cells. In contrast, suppression of Uev1A had no effects on the Rad51 foci formation or susceptibility to UV, again indicating that Uev1A is not involved in DNA repair. Interestingly, Ubc13 and Uev1A, but not Mms2, are shown to be involved in an endotoxin-mediated NF- κ B signaling pathway. In summary, we have demonstrated here that Ubc13-Uev1A and Ubc13-Mms2 have distinct biological functions and that the mammalian Ubc13-Mms2 complex is required for error-free PRR similar to its yeast counterpart.

Sequence comparison of the Uevs



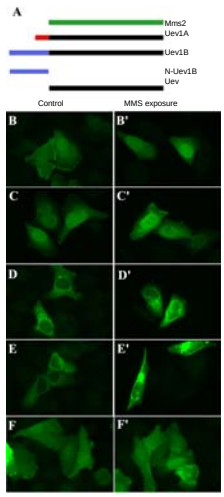
Heterologous function of Uevs in *S. cerevisiae*



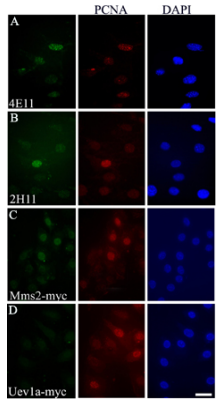
A. Expression of *hMMS2* and *UEV1A*, but not *UEV1B*, can rescue *mms2*-null *S. cerevisae* cells from killing by a DNA alkylating agent methylmathane sulfonate (MMS).
B. Yeast two-hybrid analysis demonstrates a physical interaction of Ubc13 with both Mms2 and Uev1A, but not with Uev1B.
C. GST fused -Uev1A and -Mms2 are capable of forming complexes with Ubc13.

Taken together, these results demonstrate that both Uev1A and Mms2 are capable of forming complexes with Ubc13 in vivo and in vitro, whereas Uev1B is incapable of interacting with Ubc13.

Cellular localization



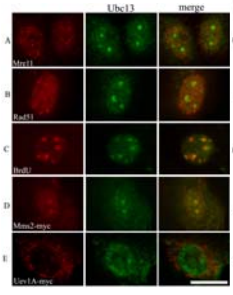
Localization to S-phase nuclei



C-terminal Myc-tagged constructs were created as indicated in the schematic representation (A) to distinguish possible localization differences of the Uevs before (B-F) and following 0.04% MMS exposure (B'-F'). Please note that the N-terminus of Uev1B is sufficient for the exclusion from the nucleus.

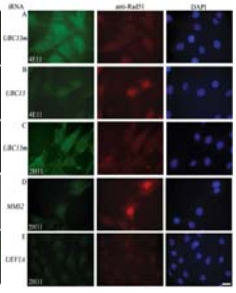
A mild detergent extraction (0.02% Triton X-100 on ice) was used to remove soluble protein before fixation. Immunocytochemistry shows a retention of Ubc13 and Mms2-myc in PCNA-positive nuclei. This suggests the formation of insoluble nuclear complexes specifically during S-phase.

Damage-induced nuclear foci formation



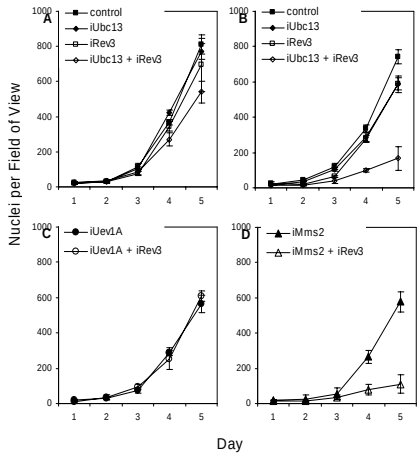
Immunocytochemistry following high-stringency detergent extraction (0.02% Triton X-100 at room temperature) of soluble protein. This demonstrates insoluble nuclear foci formation of Ubc13 and myc-tagged Mms2 colocaizing to likely sites of replication forks in response to camptothecin exposure. Note that myc-tagged Uev1A does not form distinct nuclear structures.

Spontaneous nuclear foci formation



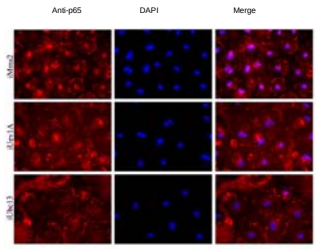
The iRNA technology was used to repress specific proteins. Immunocytochemistry shows an increase in spontaneous DNA damage (indicated by Rad51 localization) in response to the reduction of either Ubc13 or Mms2. Note that Rad51 foci were not induced in response to iRNA directed against Uev1A or a control construct (UBC13m).

Combined activity of Ubc13-Mms2 and Rev3



3T3 cells show a reduced proliferation ability in response to uv light following suppression of both Ubc13 with Rev3 (B) or Mms2 with Rev3 (D), but not Uev1A with Rev3.

Ubc13-Uev1A dependent NFkB signailing.



iRNA technology demonstrates that LPS-induced NFkB activation is dependent upon the presence of Ubc13 and Uev1A, but not on Mms2. This describes a distinct activity of Ubc13 in the presence of the alternative Uevs.

Conclusions

1. hMms2 and hUev1A can interact with yeast Ubc13 and substitute function with respect to PRR repair.
2. The N-terminal of Uev1B is sufficient for its restriction from the nucleus, and therfore unlikely to function in PRR repair.
3. Ubc13-Mms2 form nuclear complexes during S-phase and following DNA strand breaks distinct from Uev1A localization, suggesting Uev1A has alternative roles than DNA metabolism.
3. Spontaneous DNA damage in response to reduced Ubc13 or Mms2 levels suggests a critical function of Ubc13-Mms2 in DNA replication
3. Reduced proliferation in response to uv exposure following Ubc13/mms2 and Rev3 reduction strongly suggests the Ubc13-Mms2 complex is involved in DNA PRR. The inability of Uev1A to enhance uv sensitivity suggests Ubc13-Uev1A is not involved in DNA PRR.
4. Repression of either Ubc13 or Uev1A (but not mms2) represses LPS-induced NFkB signaling.

This research describes alternative activities of Ubc13 in mamallian cells, specifically Ubc13-Mms2 is involved in DNA PRR and Ubc13-Uev1A is involved in NFkB signaling.

Acknowledgments

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