Nuclear dynamics and *in vivo* functions of human Rev3

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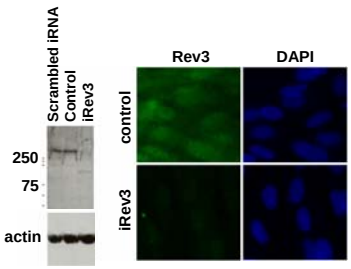
Abstract

Translesion synthesis (TLS) is a mechanism that utilizes low-fidelity polymerases during DNA replication to bypass DNA damage that otherwise blocks replicative polymerase progression. TLS is thought to occur in two steps. First, a TLS polymerase such as Polη or Rev1 inserts bases across from the damaged DNA sequence. Second, the insert is extended using the error-prone Polζ before the replicative polymerases may proceed. In order to determine the role of Polζ in TLS, we produced a polyclonal antiserum to the C-terminus of hRev3, the catalytic subunit of Polζ. Rev3 was found to be principally a nuclear protein and exposure to UV-light was sufficient to induce stable nuclear Rev3 focus formation coinciding with both Rev1 and PCNA. Using specific cell lines lacking either Rev1 or Polη, we demonstrated that although UV-induced Rev3 focus formation is dependent on Rev1, it is independent of but co-localizes with Polη. Interestingly, over-expression of Polη-GFP is capable of inducing Rev3 focus formation in the absence of exogenous DNA damage. To test the functionality of Rev3 we utilized interference RNA technology. While individual repression of either Rev3 or the parallel error-free DNA damage tolerance pathway enzyme Ubc13 resulted in little enhanced UV sensitivity, co-repression of both pathways resulted in a markedly increased UV susceptibility. Together, this data supports the hypothesis that human Polζ operates as a TLS polymerase during DNA damage tolerance in human cells.

Immunochemistry indicates Rev3 is largely a nuclear protein



Western blotting HCT116 cell extract demonstrates anti-Rev3 immunoreactivity (IR) predominates at a molecular weight far exceeding 250kD (left panel). IR on normal fibroblast cells demonstrates Rev3 predominately localizing in the nuclei (right panel). In both cases IR is suppressed using iRNA technology.

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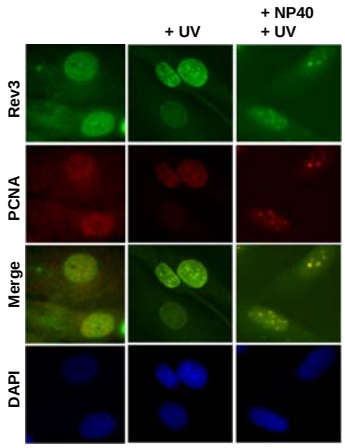
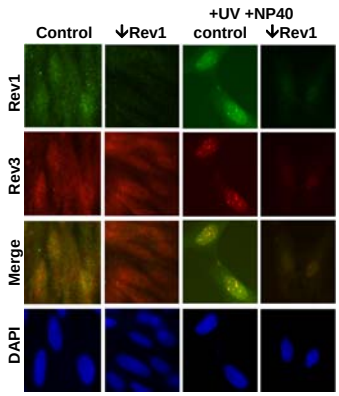
UV-induced Rev3 foci co-localizes with PCNA



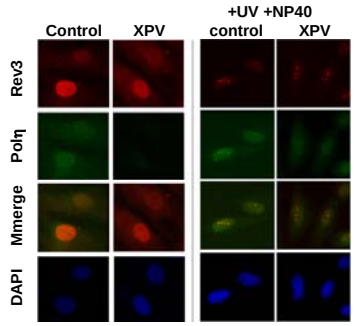
In normal, low passage fibroblasts PCNA positive nuclei are also Rev3 positive (first two columns). Following UV exposure (4J/cm^2) and NP-40 pre-extraction before fixation, UV-induced Rev3 nuclear foci co-localize with UV-induced PCNA nuclear foci (third column).

UV-induced Rev3 focus formation is dependent on Rev1



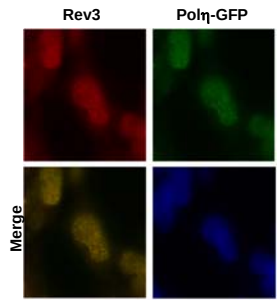
The Rev1 level is down-regulated by using ribozyme technology without affecting the level of Rev3 (left two columns). Following UV exposure and NP-40 pre-extraction before fixation, UV-induced Rev3 foci are greatly diminished in the absence of Rev1. Anti-Rev1 IR has been electronically enhanced due to low fluorescence.

UV-induced Rev3 foci co-localize with but are independent of Polη



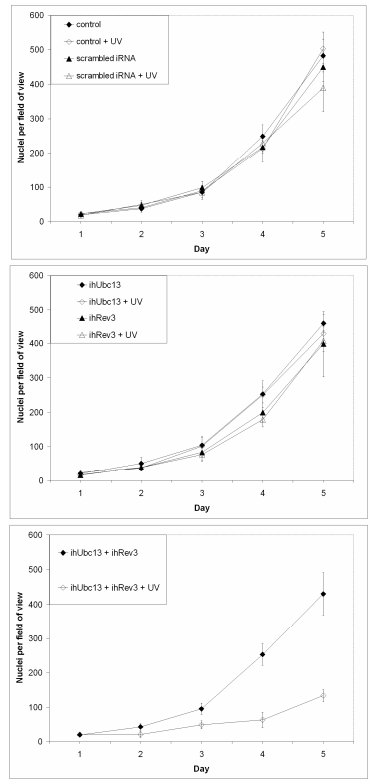
XPV cells are deficient in full length Polη, but exhibit no alteration in Rev3 IR (left two columns). Following UV exposure and NP40 pre-extraction before fixation, the formation of UV-induced Rev3 nuclear foci can be observed in XPV cells similarly to matched control cells (right two columns).

Over-expression of Polη induces spontaneous Rev3 focus formation



HEK-293-FlpN cells were transfected with Polη-GFP and exhibited spontaneous nuclear GFP focus formation (top right). Coinciding with Polη-GFP are Rev3 foci (top left) in the absence of exogenous DNA damage.

Co-suppression of Rev3 and Ubc13 exacerbates UV sensitivity



Rev3 and Ubc13 are proposed to operate in the error-prone and error-free parallel pathways, respectively, for DNA damage tolerance. To determine the functional activity of Rev3, Rev3 and Ubc13 were repressed simultaneously using iRNA. Following sub-lethal UV exposure, repressing either Ubc13 or Rev3 alone did not alter the growth characteristics of the cell populations (top two graphs). However, cells became more susceptible to UV exposure following simultaneous suppression of the two pathways (bottom graph). Repression was confirmed using ICC (not shown).

Conclusions

1. We have developed an antibody directed from the C-terminal of human Rev3. IR demonstrates that this antibody is specific for human Rev3 and this protein predominates in the nucleus.
2. Sub-lethal UV exposure induces Rev3 focus formation, which coincides with PCNA, Rev1 and Polη.
3. Although UV-induced Rev3 foci are dependent on Rev1, formation is independent on Polη.
4. Over-expression of Polη spontaneously induces Rev3 focus formation, suggesting activity in the absence of exogenous DNA damage.
5. Co-suppression of Ubc13 and Rev3 increases susceptibility to UV exposure, suggesting two independent activities in the DNA damage tolerance pathway.