SEQUENTIAL ASSEMBLY OF ENDOGENOUS HUMAN TRANSLESION DNA POLYMERASES

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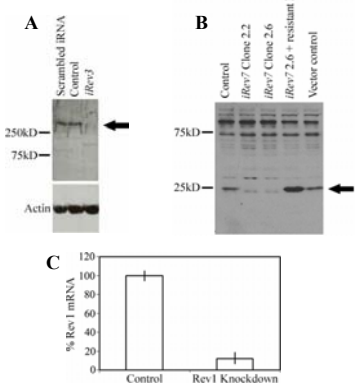
Abstract

DNA damage tolerance (DDT) is a mechanism cells utilize to replicate past DNA damage that is normally insurmountable by the high fidelity replicative polymerases, allowing continuous genome duplication in the presence of damage. In yeast, DDT operates in two parallel pathways identified as being either error-prone by utilization of the potentially mutagenic low fidelity translesion synthesis (TLS) polymerases, or error-free by DNA replication using a non-damaged DNA template with poorly characterized mechanisms. TLS has been proposed to occur as a multi-step scheme in response to a stalled replication fork such that the Y-family polymerases first insert few bases opposite the damaged template, followed with elongation by the B-family polymerase pol ζ before synthesis can be continued by the replicative polymerases. By utilizing an antibody we created to the Rev3 catalytic subunit of the B-family TLS polymerase pol ζ and combined approaches to suppress endogenous Y-family polymerases we were able to dissect the endogenous TLS polymerase switch model in cultured human cells. We found that upon UV irradiation Rev1, pol η and Rev3 are recruited to nuclear foci containing PCNA. While recruitment of Rev1 and pol η occurs independently of one another and of Rev3, the recruitment of Rev3 to the damage-induced nuclear foci requires the presence of Rev1 but not pol η. These observations support the current polymerase switch model in which pol ζ operates downstream of the Y-family polymerases in response to genomic stresses.

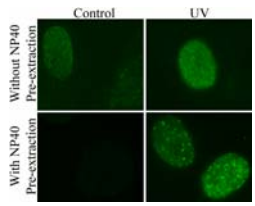
Characterization of target knockdowns



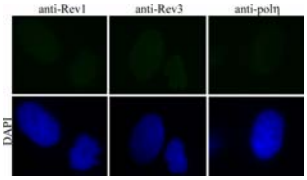
Specificity of the iRNA constructs is demonstrated by Western blotting of HCT116 cells for Rev3 (A) and MSU1.2 cells (and derivative cell lines) for Rev7 (B). Arrows in A and B indicate the expected migration distance of the target protein. MSU1.2 cells and the iRev7 derivatives and anti-Rev7 antibody were kindly provided by Dr. J McCormick, Michigan State University . Reduction Rev1 mRNA in control NF1604 cells and the ferivative Rz20 cell line using ribozyme technology is demonstrated by RT-PCR (C).

Identification of UV-inducible nuclear foci

1. Media is removed from cells grown on cover slips and are exposed to 4 J/m^2 ultraviolet light. The identical media is replaced and incubation is continued for an additional 4 hours.
2. Cover slips are rinsed with ice cold PBS and incubated with 0.4% NP40 on ice for 40 minutes with occasional agitation.
3. Cover slips are fixed in freshly prepared methanol-free 4% formaldehyde for 30 minutes.
4. After blocking, primary antibodies are applied overnight at room temperature and standard immunochemistry procedures are followed.

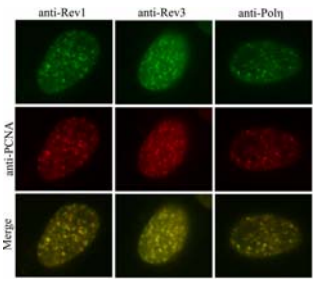


Four hours after UV exposure (4 J/m2) an increase in nuclear TLS polymerases is observed (in this case Rev3) in normal human fibroblasts (GM08402), however distinct nuclear foci are not definable without detergent (NP40) pre-extraction before fixation.



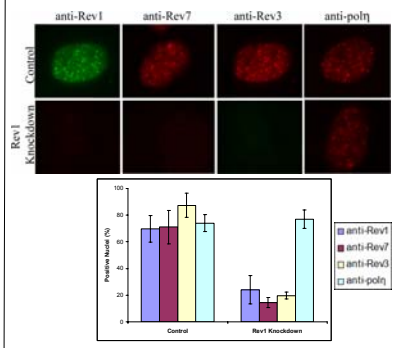
As with Rev3 foci, when cells are not exposed to UV light nuclear foci containing Rev1, Rev7 or polη are observed in less than 5% of nuclei. Corresponding nuclei are stained with DAPI.

UV-induced foci formation co-localizes with PCNA



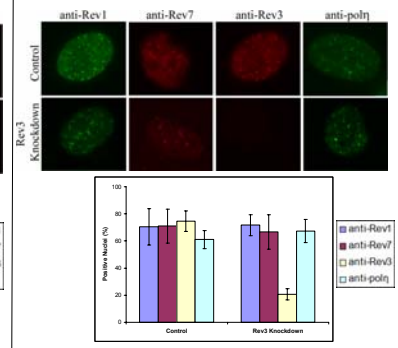
In normal human fibroblasts (GM08492) four hours after UV exposure (4 J/m^2) nuclear foci immunoreactive for Rev1, rev3 and polη are observed to co-localize with PCNA nuclear foci, suggesting an interaction as expected.

UV-induced foci formation in the absence of Rev1



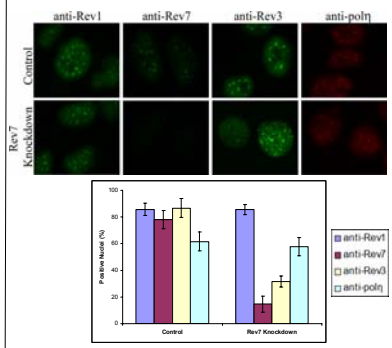
In NF1604 cells, reduction of Rev1 results in reduced nuclear UV-induced Rev7 and Rev3 foci, but not polη foci. Graphs represent the average and standard deviations of three independent experiments which exhibited reduction of Rev1 by ICC.

UV-induced foci formation in the absence of Rev3



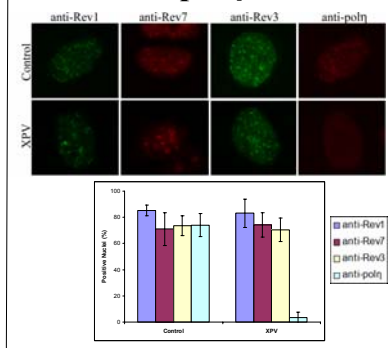
In NF1604 cells, reduction of Rev3 does not result in changes in UV-induced Rev, Rev7 or polη foci. Graphs represent the average and standard deviations of three independent experiments which exhibited reduction of Rev3 by ICC.

UV-induced foci formation in the absence of Rev7



In MSU1.2 cells, reduction of Rev7 (clone 2.6) results in a partial reduction of UV-induced Rev3 foci, but no reduction in Rev1 or polη foci. Graphs represent the average and standard deviations of three independent experiments which exhibited reduction of Rev7 by ICC.

UV-induced foci formation in the absence of polη



In XPV, cells which lack full length polη, no changes in UV-induced foci formation by Rev1, Rev7 or Rev3 are observed compared to control cells (GM08402). Graphs represent the average and standard deviations of three independent experiments.

Summary

Foci formed \ Required

	Rev1	Rev7	Rev3	polη
Rev1		No	No	No
Rev7	Yes		No	No
Rev3	Yes	Partially		No
polη	No	No	No	

Conclusions

1. Rev1, Rev7, Rev3 and polη form NP40-resistant nuclear foci in response to UV exposure.
2. UV-inducible Rev1, Rev3 and polη nuclear foci co-localize with PCNA.
3. Rev7 and Rev3 foci are dependent upon the presence of Rev1,bhowever Rev 3 may also form foci independently of Rev7.

This supports a model in which polζ operates downstream of the B-family polymerases in response to genomic damage and also suggests that Rev3 may have an additional function independent of Rev7.

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