Rev3 and DNA Damage Tolerance

Parker L. Andersen, Fang Xu, Barry Ziola, Wei Xiao.

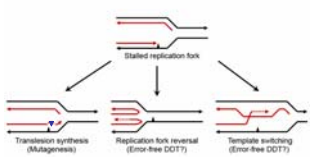
Department of Microbiology and Immunology, University of Saskatchewan, Canada. Department of Biology, Ningxia Medical College, China. Department of Pathology, University of Saskatchewan, Canada.

Abstract

Cancer is genetic disease caused by mutation. Hence, it is important for cells to minimize mutations in order to reduce the cancer incidence. However, dividing cells also have the absolute requirement to completely duplicate their genome even in the presence of DNA damage as roadblocks. It is hypothesized that mammalian cells contain a DNA damage tolerance (DTT) response to bypass these roadblocks with the possible consequence of introducing errors into the DNA and therefore promoting tumor formation. Initial studies in yeast identified a DNA polymerase Rev3 capable of translesion DNA synthesis with low fidelity, which is responsible for the vast majority of DNA damage-induced mutations. Our laboratory first reported a human counterpart of this gene, hREV3 and we have produced an antibody that allows us to specifically examine hRev3 in cultured mammalian cells. We find that hRev3 is localized to sites of DNA synthesis in response to DNA damage. Reduced expression of Rev3 results in genome instability as seen by poorly segregated nuclei. Finally, repression of Rev3 with simultaneous repression of a parallel (error-free) pathway of DDT results in enhanced susceptibility to DNA damage. These results support the hypothesis that hRev3 is involved in DDT in mammalian cells. Many mutations are not directly caused by the damage to the DNA; rather, active processes in response to this damage by the cell may contribute to incorporation or fixation of mutations. We propose that Rev3 plays such a role and hence is responsible for genome instability.

Introduction

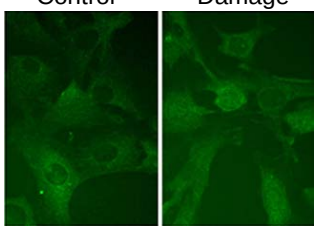
In yeast, mutagenesis is largely dependent on Rev3, a DNA polymerase intended to bypass DNA damage during it's duplication. This activity is thought to allow complete chromosome segregation in the presence of DNA damage, which is essential for cell survival. However, Rev3 has the misfortune on introducing and stabilizing DNA mutations which may allow the progression of cancer. Here we hypothesize that the putative human Rev3 operates in error-prone DDT.



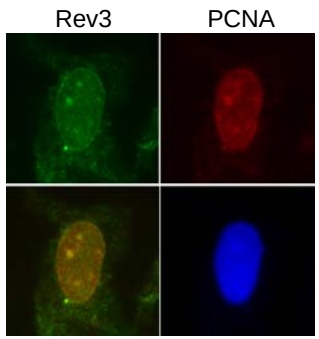
Proposed DDT pathways at the replication fork.

Results

Rev3 localizes to sites of DNA synthesis following damage



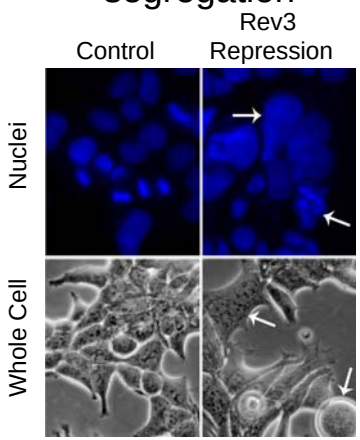
In response to ultraviolet light, Rev3 relocalizes to the nuclei.



An enhanced technique and higher magnification reveals that UV exposure induces the co-localization of Rev3 with PCNA (proliferating cell nuclear antigen). PCNA is an indicator of sites of DNA synthesis.

This supports the hypothesis that hRev3 is involved in DDT.

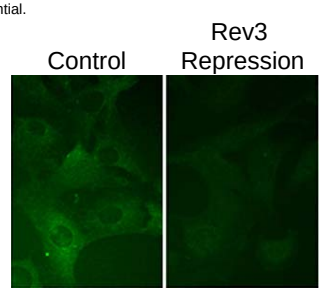
Rev3 is required for proper chromosome segregation



Chronic repression of Rev3 protein levels results in cells with abnormal nuclei and poor chromosome segregation (arrows).

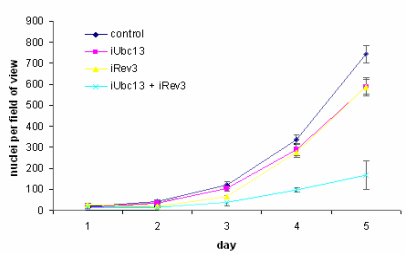
This suggests Rev3 is required for normal DNA synthesis.

Eventually these cells become non-viable, suggesting Rev3 is essential.



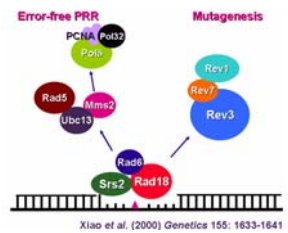
A technique called interference RNA can be used to specifically repress protein expression, in this case iRev3

Rev3 operates in a parallel pathway with error-free DDT



The growth curve describes the proliferation activities of cells in culture. In this case simultaneous suppression of both the error-prone (Rev3) and error-free (Ubc13) pathways (see below) results in a decreased duplication rate following DNA damage. Repression of either pathway alone has very little effect on cell duplication rate. As a control, suppression of Uev1A did not augment growth rate (data not shown for clarity).

This supports the postulation of parallel DDT pathways in mammalian cells as characterized in yeast.



The two DDT pathways characterized in yeast.

Conclusions

1. Human Rev3 responds by localizing to nuclear foci in response to DNA damage. Additionally, Rev3 co-localizes with supplementary polymerases which have been demonstrated to exhibit translesion synthesis activities (data not shown).
2. Rev3 is required for genome segregation as observed by the appearance of large, irregular nuclei and misaligned chromosomes following it's repression.
3. Simultaneous suppression of Rev3 and Ubc13 (or Mms2, data not shown) results in a greater susceptibility to damage and demonstrates DDT is operational in mammalian cells.

Implications

1. The localization of Rev3 to sites of DNA damage following injury strongly suggests that this enzyme is involved in a DNA damage response. Because of the low stringency of yeast Rev3, we can further postulate that human Rev3 is an active player in promoting mutagenesis.
2. Because nuclear segregation is compromised in cells chronically depleted in Rev3 we can postulate DNA synthesis is incomplete. Rev3 appears to be essential during scheduled DNA synthesis.
3. The additive effects of suppressing Rev3 and the error-free pathway of DDT strongly supports the concept that either pathway can bypass DNA lesions during synthesis. This suggests that repression of the error-free pathway may induce enhanced use of the mutagenic polymerases and promote cancer progression.
4. The existence of an error-prone DDT pathway dependent upon Rev3 supports the suggestion that mutagenesis is largely an active process carried out by the cell.

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