Tolerance of DNA Replication Stress Is Promoted by Fumarate Through Modulation of Histone Demethylation in *Saccharomyces cerevisiae*

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Abstract

Our study uncovers links between the metabolic enzyme fumarase plus the metabolite fumarate and chromatin modifications during DNA damage response in *Saccharomyces cerevisiae*. In mammalian cells, fumarase becomes enriched at sites of double stranded break (DSB) through interaction with the histone variant H2A.Z. At DSBs, fumarase promotes DNA repair by nonhomologous end joining (NHEJ) by local production of fumarate which in turn acts as an inhibitor of KDM2B, a H3 K36-specific histone demethylase. We have demonstrated that treatment with hydroxyurea (HU), which creates stalled replication forks by depletion of nucleotides, leads to upregulation as well as nuclear enrichment of Fum1p. We have also shown that increased cellular levels of fumarate (upon deletion of *FUM1* or addition of exogenous fumarate) suppresses the sensitivity to HU in *htz1*Δ mutants in a manner that is independent of modulating nucleotide levels. In fact, fumarate confers resistance to HU in *htz1*Δ mutants by inhibition of the H3 K4-specific histone demethylase Jhd2p, and increasing H3 K4 methylation levels. Sensors and mediators of the DNA replication checkpoint were required for fumarate-dependent resistance to HU in *htz1*Δ whereas factors involved in processing of regressed replication forks were dispensable. Together, our findings imply that high cellular levels of fumarate support processing of replicative intermediates by regulation of histone methylation, thereby promoting genome integrity.

Introduction

- Fumarase is a TCA cycle enzyme that catalyzes the reversible conversion of fumarate to malate. Fumarase is abundant in both mitochondria and cytoplasm.
- Fumarase also acts as a tumor suppressor, and defects in the gene encoding fumarase (FH) are commonly found in hereditary leiomyomatosis and renal cell cancer, glioblastomas and neuroblastomas.
- Loss of fumarase leads to accumulation of intracellular fumarate
- Fumarate is an inhibitor of Jumonji domain-containing histone demethylases
- In mammalian cells, local generation of fumarate enhances H3 K36 methylation and promotes NHEJ
- Mammalian H2A.Z and the budding yeast ortholog Htz1p can promote DNA repair by
- NHEJ as well as homologous recombination
- Deletion ofFUM1in yeast, and loss of the catalytic activity of fumarase in human cells, or loss of function mutations in fumarase in HLRCC tumors, cause accumulation of fumarate to high cellular levels (several hundred-fold increase in yeast, millimolar levels in humans)

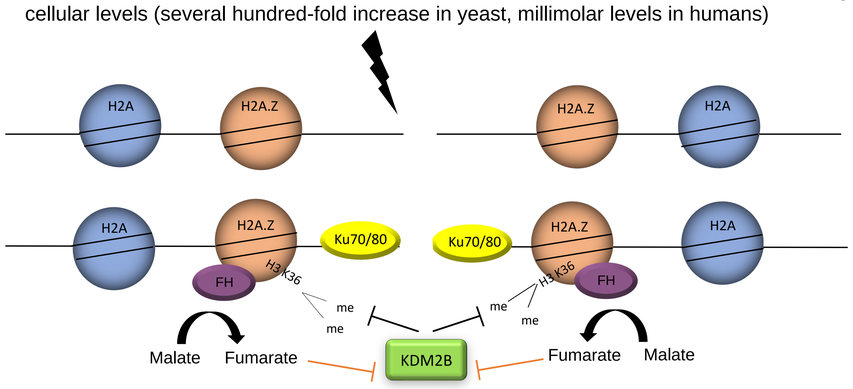


Figure 1. Upon DNA damage, fumarase (FH) is recruited to sites of double stranded break (DSB) through interaction with the histone variant H2A.Z. Fumarate generated by DSB-associated FH acts as an inhibitor of α-ketoglutarate-dependent histone demethylase KDM2B causing an increase of H3 K36 methylation at DSBs. This, in turn, promotes binding of Ku70/80 to DSB ends and enhances DSB repair by NHEJ.

Results

Fumarase is induced and becomes enriched in the nuclear fraction upon exposure to hydroxyurea (HU)

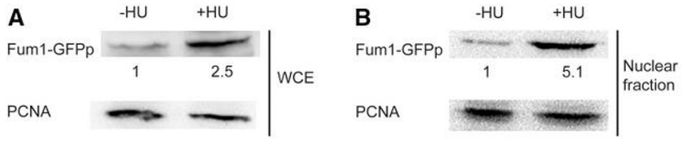


Figure 2. Yeast expressing Fum1-GFPp were incubated in the absence or presence of 200 mM HU at 30°C for 3 hr. Whole cell extracts (A), or nuclear fractions (B) were analyzed by immunoblotting using anti-GFP, and anti-PCNA antibodies. A representative immunoblot and fold enrichment of Fum1p from two independent experiments is shown. Levels of Fum1-GFPp were normalized to levels of PCNA (loading control), then expressed relative to signal that was observed in the absence of HU, which was set to 1.

Sensitivity of *htz1Δ* mutants to DNA replication stress caused by HU is suppressed by high cellular levels of the metabolite fumarate (achieved by addition of exogenous fumarate or deletion of Fumarase, Fum1p)

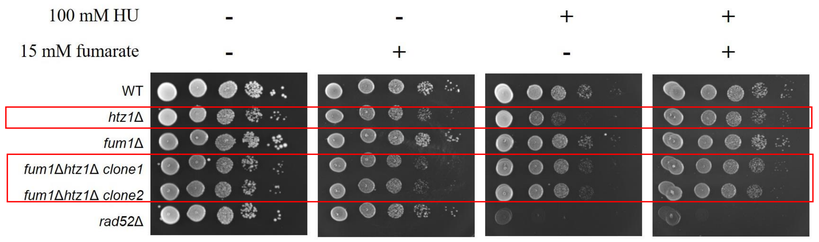


Figure 3. Cells with genotypes as indicated were grown overnight in rich (YPD) medium, then 3 ul of 10-fold serial dilutions were spotted onto YPD medium containing the indicated concentrations of fumarate and/or HU, and incubated at 30°C for 2 days prior to imaging.

YKu70p is not required for suppression of sensitivity of *htz1Δ* mutants to DNA replication stress by fumarate.

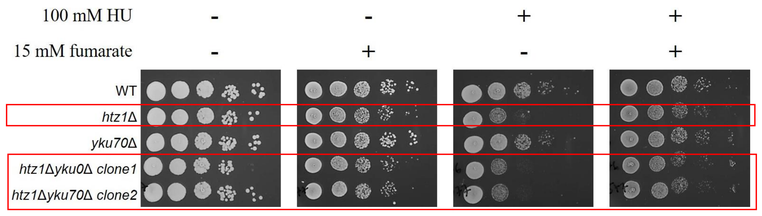


Figure 4. Strains with indicated genotypes were analyzed in serial dilution growth assays as described in Figure 3

Loss of JmjC domain-containing histone demethylase Jhd2p suppresses the sensitivity to DNA replication stress of *htz1Δ* mutants.

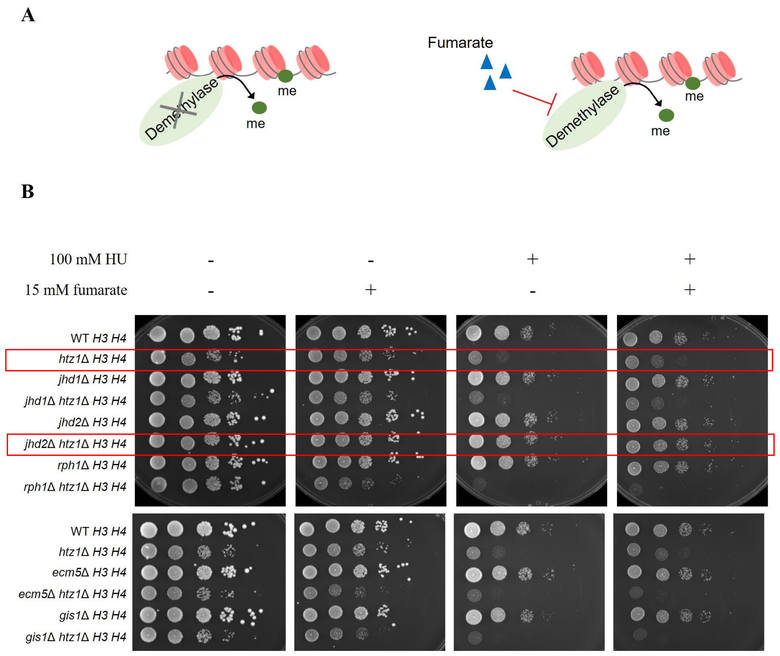


Figure 5. (A) Enhancing histone methylation by deletion of histone demethylase(s) or enzyme inhibition by fumarate. (B) Genetic interaction analyses between *htz1*Δ mutants and histone demethylase mutants. Strains with indicated genotypes were analyzed in serial dilution growth assays as described in Figure 3.

Fumarate modulates levels of JDH2-dependent H3 K4 methylation.

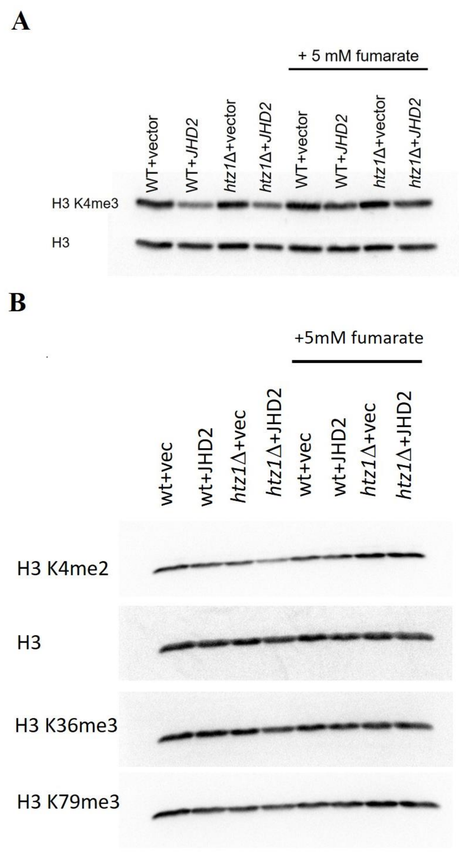


Figure 6. Wild-type and *htz1*Δ strains carrying an empty vector or a plasmid for overexpression of Jhd2p were grown logarithmically in selective medium with or without 5 mM fumarate. Whole cell extracts were analyzed by immunoblotting against A) H3 K4me3 and H3 (loading control) or B) H3 K4me2, H3 K36me3 or H3 K79me3 or H3 (loading control)

Impact of loss of HTZ1 and exogenous fumarate on sensitivity to DNA replication stress of mutants with defects in DNA replication checkpoint and processing and restart of aberrant replication forks.

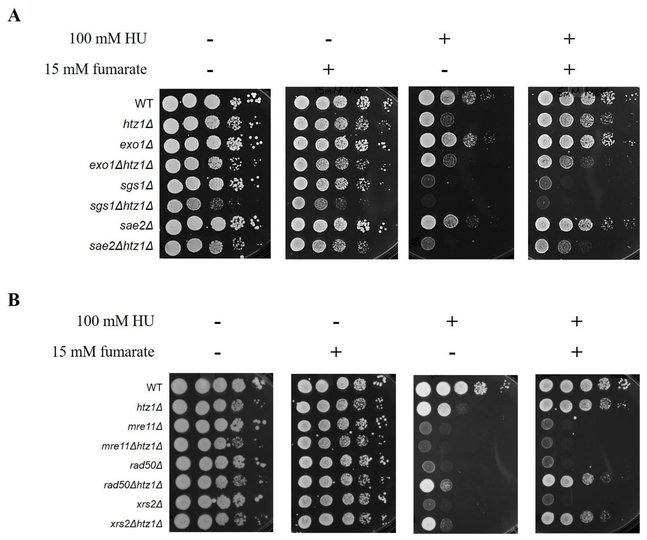
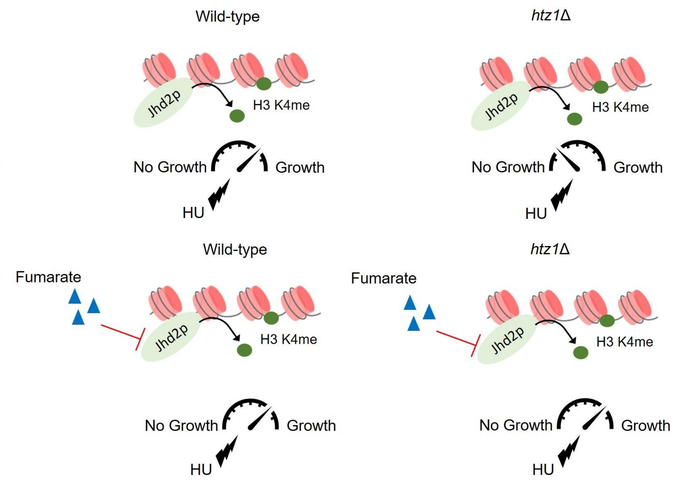


Figure 4. (A) Fumarate suppresses the sensitivity to DNA replication stress in cells lacking EXO1,orSAE2in the presence or absence of *HTZ1*. (B) Loss of *HTZ1* confers resistance to DNA replication stress of cells lacking subunits of the MRX complex, and fumarate enhances this effect. Strains with genotypes as indicated were analyzed as described in Figure 3.

Model

Fumarate promotes growth upon DNA replication stress in *htz1Δ* mutants by inhibition of Jhd2p and promoting high levels of H3 K4 methylation



Conclusions

- Yeast fumarase and fumarate act as DNA damage response factors
- High cellular levels of fumarate (upon deletion of *FUM1* ,or addition of exogenous fumarate) relieves the sensitivity to DNA replication stress of yeast lacking *HTZ1*
- High cellular levels of fumarate suppresses the DNA damage sensitivity of *htz1*Δ and this suppression in independent of the NHEJ pathway but requires components of the intra-S phase checkpoint
- Deletion of *JHD2* also suppresses the DNA damage sensitivity of *htz1*Δ mutants
- Our genetic studies show that fumarate confers resistance to HU by modulation of histone methylation levels through inhibition of Jhd2p

The results of this study has been published below:

Saatchi F, Kirchmaier A. L. "Tolerance of DNA replication stress is promoted by fumarate through modulation of histone demethylation and enhancement of replicative intermediate processing in *Saccharomyces cerevisiae*" GENETICS. 2019, 212: 631–654.