GEN1 In Processing Replication And Recombination Intermediates

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Molecular Biology and Biotechnology (Intensive)
Research Colloquium for Science UG Students 2021-22

Abstract

Holliday junctions (HJ) are four-way DNA junctions that are formed during Homologous recombination. MUS81 and GEN1, structure-selective endonucleases, are HJ resolvase that are independently involved in the Resolution pathway, ensuring proper chromosome segregation. The N-terminal fragment (1-527a.a.) of human GEN1 contains the nuclease and DNA binding domains. However, the function of the C-terminus, although very important, is not clearly known. In this study, several truncations of GEN1 in the C-terminus have been generated and used to rescue GEN$^{-/-}$ cells in absence of MUS81 to identify its functional region. Furthermore, GEN1's involvement in late replication intermediates (LRIs) is also studied by observing hypersensitivity of GEN$^{-/-}$ cells to replication stress in several GEN$^{-/-}$ cell lines.

Introduction

MUS81 and GEN1 are synthetically lethal; therefore, only the GEN1 construct with the functional region in the C-terminus can rescue the cells in absence of MUS81. It was seen that GEN1 1-780aa fragment could not rescue the cells.(Figure 1)

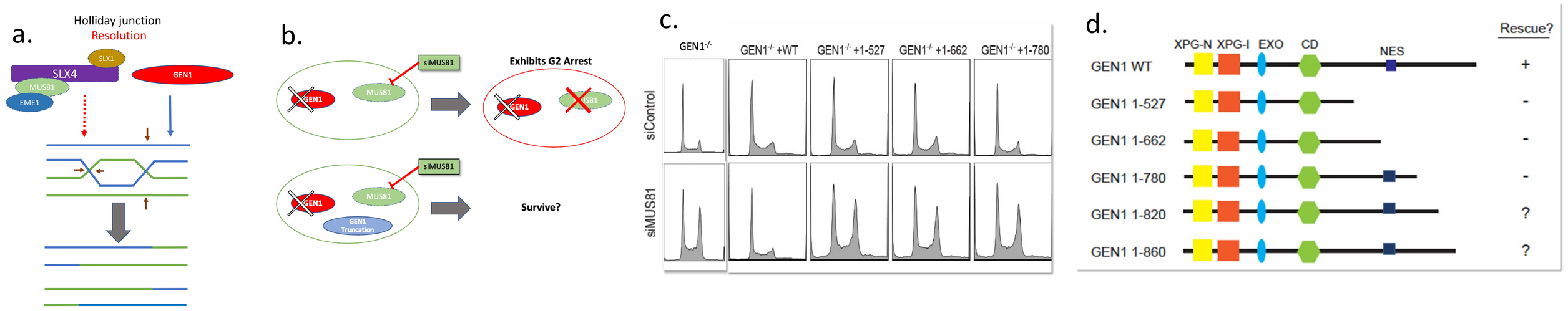


Figure 1. GEN1 and MUS81 exhibit synthetic lethality and GEN1$^{-/-}$ cells can not be rescued by the N-terminal fragment of GEN1 when MUS81 is depleted. a) Schematic diagram of Holliday junction resolution pathways b) Schematic representation of synthetic lethality of GEN1 and MUS81 c) WT and respective cells were treated with siMUS81 and siControl, FACS results show their DNA content distributions. D) A schematic diagram for the GEN1 truncations and their ability to rescue the GEN$^{-/-}$ cells treated with siMUS81

Moreover, **GEN1's LRI processing *in vivo*** has been hinted by the observation of its capability to cleave replication fork and frap structures in vitro.[1-2] Depletion of MUS81 leads to the formation of CFS-associated anaphase bridges, indicating that MUS81-mediated cleavage of LRIs unlinks the sister chromatids.[3-4] Moreover, GEN1-dependent DSB occurrence has been reported upon pathological replication stress.[5] Therefore, we hypothesize that GEN1 processes un-replicated DNA to allow proper chromosome segregation.

Research Aims

1. To pinpoint the functional region of GEN1 in the C-terminus
2. To pinpoint the post-translational modification that occurs in the functional region.
3. To establish that GEN1$^{-/-}$ cells are sensitive to replication stress under Aphidicolin treatment and Hydroxyurea treatment
4. Investigate if GEN1 resolves un-replicated DNA and involved in promoting mitotic DNA synthesis

Methods and Results

1. Generating GEN1$^{-/-}$ + GEN1 1-820 and GEN1$^{-/-}$ + GEN1 1-860 Stable Cell Lines

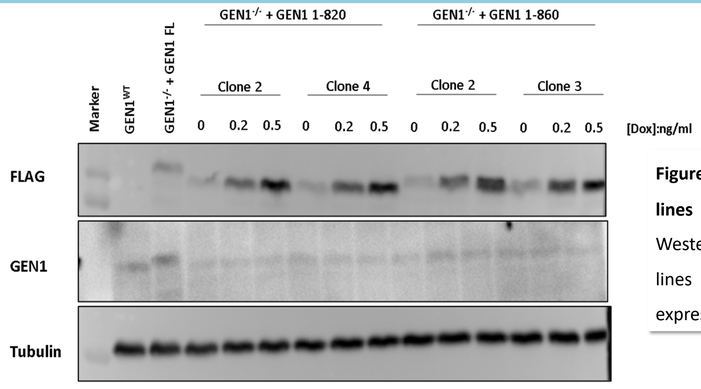


Figure 2. Successful Generation of Stable GEN$^{-/-}$ cell lines with GEN1 1-820 and 1-860 Truncations. Western Blot of successful colonies of the stable cell lines with Doxycycline dosage dependent GEN1 expression

2. Testing if the new truncations can rescue the MUS81-depleted GEN1$^{-/-}$ cells

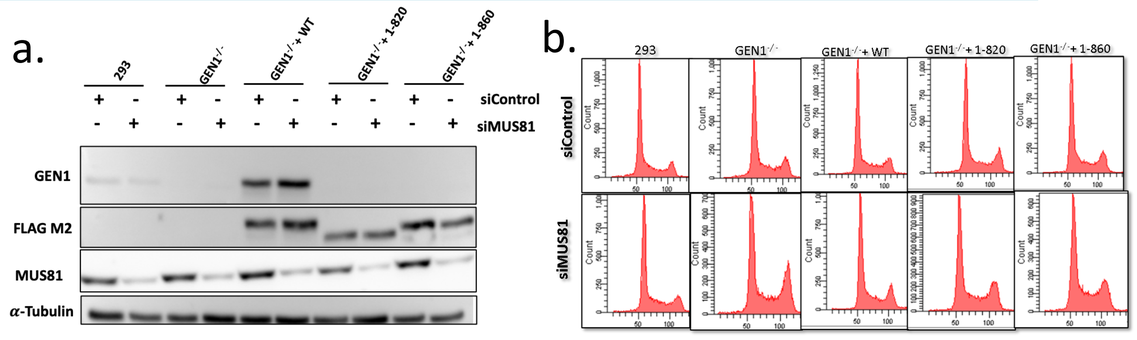


Figure 3. GEN1 1-820 and 1-860 truncation were used to rescue the cells from the synthetic lethality a) Western Blot to check the expression of GEN1 truncations using the antibodies as indicated d) FACS results show their DNA content distributions

3. Problems with MUS81 Knockdown efficiency

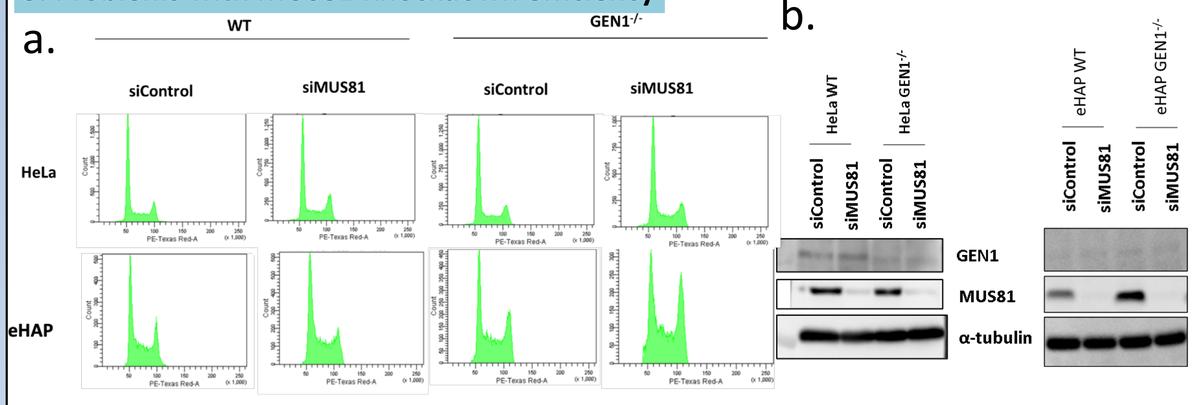
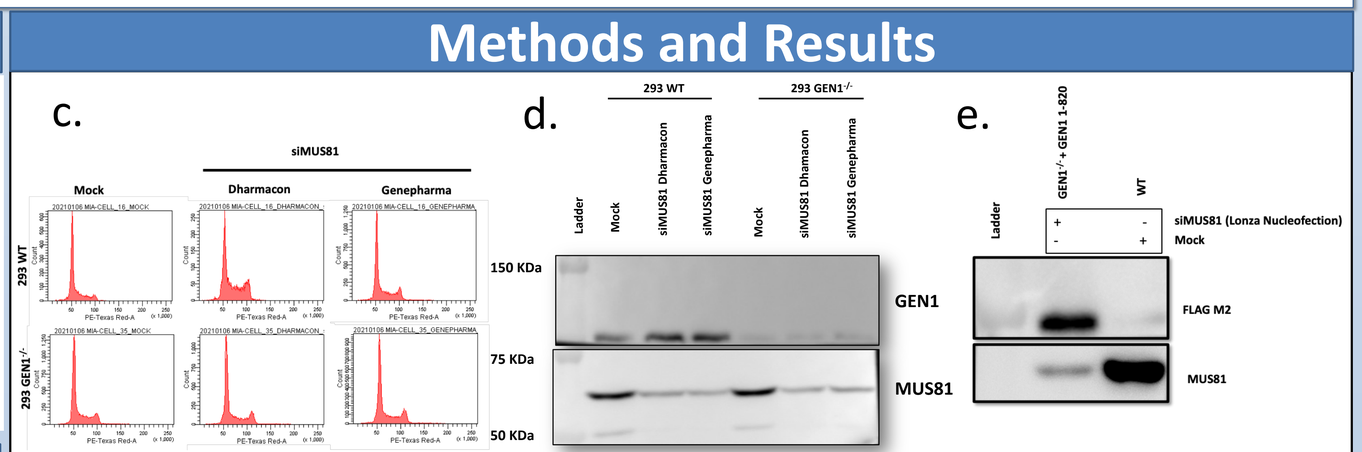


Figure 3. MUS81 knockdown efficiency in HeLa and eHAP cell lines. A) FACS results show DNA content distribution B) Western Blot to check protein expression C) FACS data for double siMUS81 treatment experiment. siMUS81 of two different companies used. D) Western Blot results of C E) Western Blot to check for MUS81 expression after siMUS81 nucleofection.

Methods and Results



4. GEN1's not Ubiquitylated or SUMOylated *in vivo*

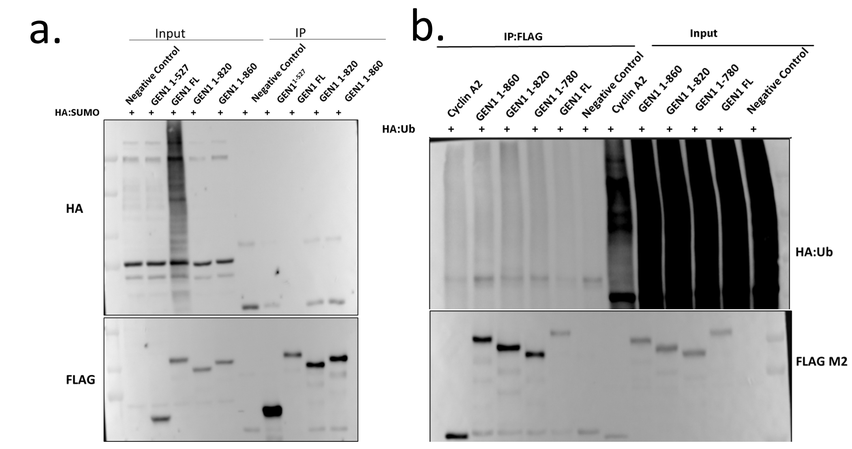


Figure 4. GEN1's not Ubiquitylated or SUMOylated *in vivo* a) Immunoprecipitation of GEN1 truncations using ANTI-FLAG® M2 affinity gel following co-transfection of the truncation and HA:SUMO. Western Blot using HA and FLAG M2 are shown. b) Immunoprecipitation of GEN1 truncations using ANTI-FLAG® M2 affinity gel following co-transfection of the truncation and HA:Ubiquitin. Western Blot using HA and FLAG M2 are shown

5. U2OS, eHAP and RPE1 GEN1$^{-/-}$ cell lines are sensitive to replication stress

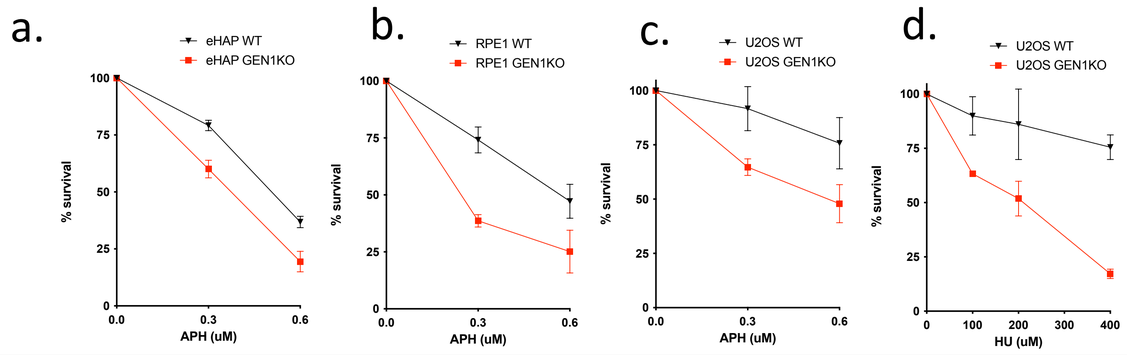


Figure 5. Survival Analysis of GEN1$^{-/-}$ in different cell lines in response to Replication stress inducing drugs, Aphidicolin, and Hydroxyurea in low doses. Clonogenic assays were carried out to investigate the survival rate. a-c respectively represent survival data for GEN$^{-/-}$ in eHAP,RPE1, and U2OS cell lines in response to Aphidicolin. d)U2OS cells' response to Hydroxyurea. P-value calculated from Two-tailed Student's t-test.

Future Steps

- To improve MUS81 knockdown efficiency
  - Generate GEN1$^{-/-}$ + GEN1 Truncation + osTIR1 +MUS81-mAID-GFP cells lines
  - Generate inducible GEN1 Truncation cell lines with eHAP cells
- Perform in vitro analysis of Ubiquitylation and SUMOylation once the functional region is identified
- Immunofluorescence experiment to investigate whether GEN1 is involved in Mitotic DNA synthesis

References and Acknowledgements

Data obtained from Dr.Gary Chan with his permission. All rights of the data belong to Dr. Chan.

***** This work was possible due to enormous support from Dr. Gary Chan, and his lab members, and the faculty of Science.**

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