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FLP1 MAY FUNCTION IN THE RESOLUTION OF RECOMBINANT DNA INTERMEDIATES

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ABSTRACT

Mus81 is a structure-selective endonuclease which constitutes an alternative pathway in parallel with the helicase-topoisomerase Sgs1-Top3-Rmi1 complex to resolve a number of DNA intermediates during DNA replication, repair, and homologous recombination. Previously, it was shown that the N-terminal region of Mus81 was required for its in vivo function in a redundant manner with Sgs1; sgs1Δmus81$_{Δ100N}$ cells are sensitive to DNA damaging agents. In this study, a single-copy suppressor screening to seek for a factor(s) that could rescue the drug sensitivity of sgs1Δmus81$_{Δ100N}$ cells was performed and revealed that Flp1, a site-specific recombinase 1 encoded on the 2-micron plasmid was a suppressor. This result suggests a function of Flp1 in coordination with Mus81 and Sgs1 to resolve the recombinant DNA intermediates.

Keywords: Mus81, Sgs1, genetic screening, homologous recombination repair, Flp1.

TÓM TẮT

Flp1 có thể tham gia vào con đường phân giải các phân tử ADN trung gian tái tổ hợp

Mus81 là một endonuclease chọn lọc cấu trúc và tạo nên một con đường song song dư thừa với phức hợp helicase-topoisomerase Sgs1-Top3-Rmi1trong việc phân giải rất nhiều phân tử ADN trung gian trong quá trình sao chép, sửa chữa và tái tổ hợp tương đồng của ADN. Phần đầu N của Mus81 đã được chứng minh là cần thiết cho Mus81 in vivo để thực hiện chức năng của protein theo con đường song song dư thừa đối với Sgs1: đột biến sgs1Δmus81$_{Δ100N}$ khiến tế bào nấm men trở nên rất mẫn cảm với các chất gây tổn thương ADN. Trong nghiên cứu này, sàng lọc nhân tố ức chế một bản sao để tìm kiếm một (hoặc nhiều) tác nhân có khả năng giải cứu tính nhạy cảm độc tố của tế bào nấm men sgs1Δmus81$_{Δ100N}$ đã được thực hiện và chỉ ra Flp1, một recombinase đặc hiệu vị trí được mã hóa trên plasmid 2-micron là nhân tố ức chế. Kết quả này thể hiện rằng Flp1 có thể tham gia cùng Mus81 và Sgs1 trong việc phân giải các phân tử ADN trung gian tái tổ hợp.

Từ khóa: Mus81, Sgs1, sàng lọc di truyền, tái tổ hợp tương đồng, Flp1.

1. Introduction

Mus81, a highly conserved DNA structure–specific endonuclease, is related to the XPF/Rad1 family of proteins involved in DNA nucleotide excision repair. Mus81 functions as a heterodimeric protein complex with a partner, namely Eme1 and Eme2 in humans, Eme1 in fission yeast, and Mms4 in budding yeast [1-3]. Its partner proteins are

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indispensable for stability and the nuclease activity of the complex [4]. The *mus81* mutants are hypersensitive to different types of DNA damaging agents including ultra violet irradiation, methyl methanesulfonate (MMS), hydroxide urea (HU), 2-phenyl-3-nitroso-imidazo [1,2-α] pyrimidine… [5, 6]. Mus81 can cleave a numerous of branched-DNA structures that may form in vivo during many DNA transactions such as nicked Holliday Junctions (HJs), D-loop, replication forks with the lagging strand at the junction point, and 3'-flap [1, 3, 7]. *MUS81* and *MMS4* genes were both identified in a synthetic lethality screen of *sgs1*Δ mutants [8]. Sgs1, a member of the ubiquitous RecQ family of DNA helicases was shown to form a stable complex with Top3 and Rmi1 which enhances the enzymatic activity of Sgs1-Top3 complex. Importantly, the synthetic lethality of double deletion of *mus81* or *mms4* together with *sgs1* can be rescued by further deletion of recombination proteins, such as Rad51 or Rad52 [1, 7, 8]. These results prove that Mus81 complex functions downstream of homologous recombination, being significantly involved in processing recombination intermediates in parallel or redundantly with Sgs1 complex [6, 9, 10].

Recently, our previous study showed the genetic and functional interaction of Rad27, an important nuclease involving in Okazaki fragment processing and base excision repair, and the Mus81 complex [11, 12]. The functional interaction of Mus81 complex and its partner depended on their physical interaction, specifically requiring the N-terminal region of Mus81 [12]. Moreover, the physical and functional interactions are significantly important for cellular function of Mus81 [12]. Here, we further investigated the significance role of Mus81 N-terminus in vivo by performing a single-copy suppressor screening to seek for a factor(s) that can suppress the cellular defect caused by function loss of Mus81 N-terminal region. Through screening, we successfully recovered a candidate that can rescue the HU sensitivity of *sgs1Δmus81*$_{\Delta 100N}$ mutant cells, namely *FLP1*, a site-specific recombinase 1 which is encoded on the 2-micron plasmid.

2. Materials and method

2.1. Yeast strains

Saccharomyces cerevisiae NJY1777 (*MATa ade2-1 ade3::hisG ura3-1 his3-11,15 trp1-1 leu2-3,112 lys2 mus81-10::KAN sgs1-20::hphMX4 can1-100 + pJM500-URA3-SGS1*) was a courtesy from Dr. Miki Ii at University of Alaska Anchorage (AK, USA) [13].

2.2. Screening single-copy suppressors of sgs1Δmus81$_{\Delta 100N}$ *mutant*

Yeast genomic DNA library was constructed by Sau3AI-partial digestion of genomic DNA of *S. cerevisiae* YPH499 strain (*MAT*a *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*). The fragmented genomic DNA with estimately 5.6 kb in length on average was ligated into BamHI-digested pRS315 plasmid, a yeast centromere vector with a *LEU2* marker. Ligation product was then transformed into *Escherichia coli* competent cells and the library plasmids were extracted and stored at -80°C for long-term usage. NJY1777 cell

containing a plasmid harboring wild-type *SGS1* gene with a *URA3* marker was transformed with *mus81$_{\Delta 100N}$* gene consisted in pRS314 plasmid, a yeast centromere vector with a *TRP1* marker. Transformants were grown in the selective media and transferred onto plates containing 5-FOA, producing double mutant *sgs1Δmus81$_{\Delta 100N}$* cells. The double mutant cells were then transformed with yeast genomic DNA library. Transformants were grown in selective media for 24 hours at 30°C, followed by replica plating onto the same medium supplemented with 20 mM HU. Selected colonies that grew on HU plates were examined for HU resistant capability by drop dilution assay. The transformants were grown on plates containing selective synthetic defined media and a single colony from each of the transformants was inoculated into liquid media (1 ml) until saturation. Cell densities were adjusted to OD_{600}=1 (~2×10^7 cells/ml) by diluting with dH_2O, followed by spotting of 10-fold serial dilutions onto selective media plates containing with or without DNA damaging agents. The plates were then incubated for 4 days at 30°C. The yeast cells that could grow on plates containing HU better in comparison to negative control were considered as HU-resistant cells. The HU-resistant colonies were transferred to liquid medium, and total plasmids were isolated. To confirm single-copy suppression, recovered plasmids were retransformed into the *sgs1Δmus81$_{\Delta 100N}$* mutant cells and examined for their ability to support cell growth in the presence of HU. Double-checked plasmids were analyzed by sequencing to identify genomic DNA fragments inserted. One of the analyzed plasmids contained the full length of *FLP1* gene.

3. Results

3.1. The single-copy suppressor screening to find out a factor(s) that can suppress the cellular defect causing by the dysfunction of N-terminal region of Mus81

We aim to seek for an alternative pathway that can cope with the loss of function of the important Mus81 N-terminal region. To perform the single-copy suppressor screening to define a suppressor of Mus81 lacking N-terminus mutant, we chose the HU sensitive phenotype of the *sgs1Δmus81$_{\Delta 100N}$* cells to identify a factor that can rescue this cellular defect. Collectively, after replica plating step, there were fifty-seven colonies that could grow on HU plates. Choosing those colonies and using drop dilution assay, we were able to examine the HU-resistant ability of fifty-four colonies (Figure 1). Among fifty-four checked colonies, forty-one were capable of suppress HU sensitivity of the *sgs1Δmus81$_{\Delta 100N}$* mutant. There were twenty-three strong suppressors in comparison to wild-type cells (Figure 1, Table 1). Next, plasmids from forty-one colonies were extracted and re-transformed into the *sgs1Δmus81$_{\Delta 100N}$* cells. Among forty-one candidate plasmids extracted, thirty-eight successfully created transformants. Then transformants were serial-diluted spotted onto plates containing HU to evaluate their survival. Among thirty-eight obtained transformants, only sixteen were capable of resisting to HU treatment (Figure 2, Table 1).

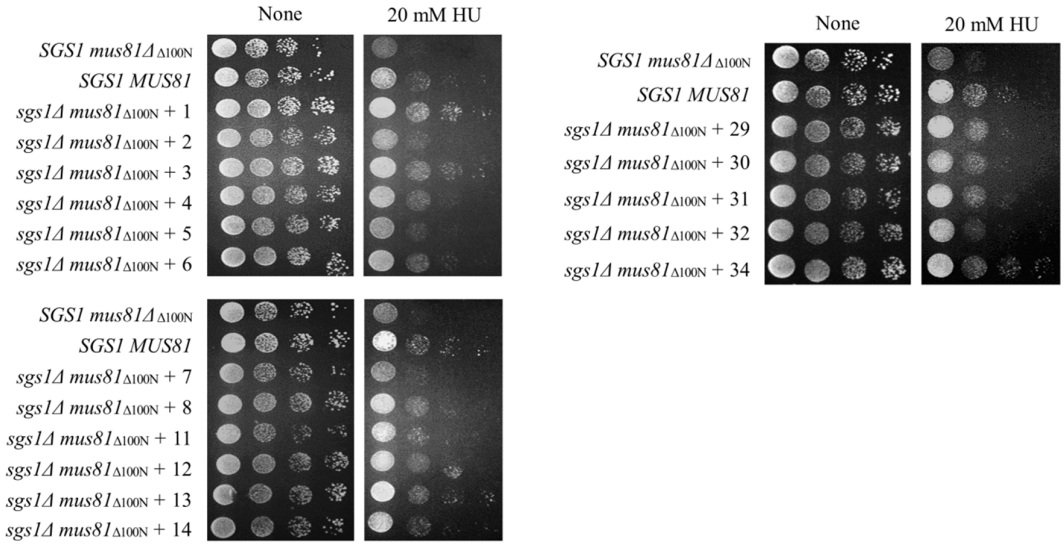

Figure 1. *A drop dilution assay examining the transformant colonies that survive in the presence of HU.* The *sgs1Δmus81$_{Δ100N}$* cells containing yeast genomic DNA fragment which survived on HU plates after replica step were selected and serial-diluted spotted onto plates without or with 20 mM HU.

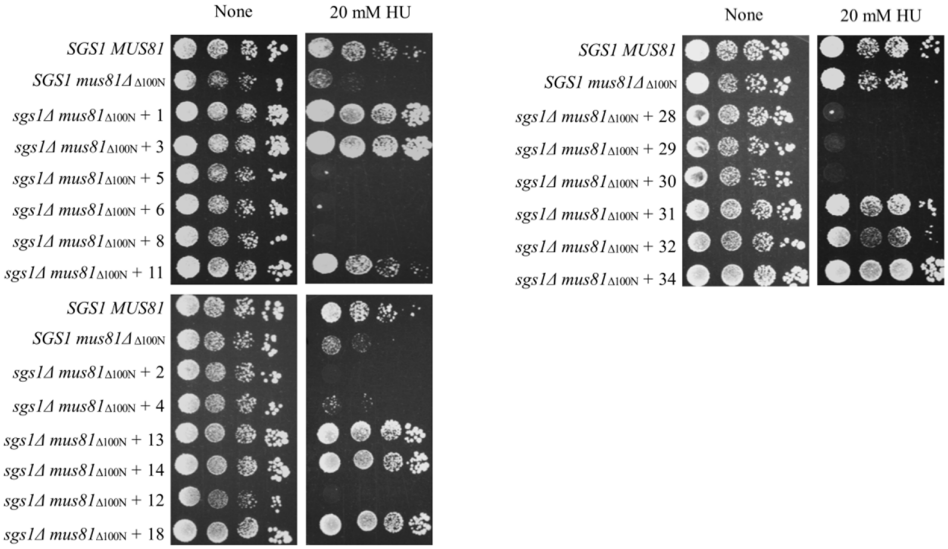

Figure 2. *A drop dilution assay to confirm the suppression ability of the candidates.* The s*gs1Δmus81$_{Δ100N}$* cells were transformed with extracting plasmids from selected candidates and then serial-diluted spotted onto plates without or with 20 mM HU.

Table 1. *Summary of the single-copy suppressor screening of sgs1Δmus81$_{Δ100N}$ mutant*

	Candidate	Suppression	Confirmed Suppression	Gene sequence
1	+++	+++	*SGS1*	
2	+	-		
3	+++	+++	*SGS1*	
4	+	-		
5	+	-		
6	++	-		
7	-	/		
8	+	-		
9	NA	/		
10	NA	/		
11	+	++	NA	
12	+	-		
13	++	+++	*SGS1*	
14	+	+++	*SGS1*	
15	-	/		
16	NA	/		
17	-	/		
18	+++	+++	*SGS1*	
19	+	-		
20	-	/		
21	+++	+++	*SGS1*	
22	-	/		
23	+++	+++	*SGS1*	
24	+++	+++	*SGS1*	
25	+	+++	*SGS1*	
26	+	-		
27	++	+++	*SGS1*	
28	+	-		
29	+	-		
30	+	-		
31	+	++	*FLP1*	
32	+	-		
33	++++	NA		
34	+++	+++	*SGS1*	
35	+	-		
36	F	/		
37	+++	+++	*SGS1*	
38	+	-		
39	+++	+++	*SGS1*	
40	+++	+++	*SGS1*	
41	-	/		
42	-	/		
43	-	/		
44	+++	NA		
45	++++	-		
46	++++	/		
47	++	-		
48	++++	-		
49	-	/		
50	+	-		
51	++++	-		
52	+++	-		
53	+++	-		
54	-	/		
55	++++	-		
56	-	/		
57	+	-		
	Sum	41	16	

(+) suppressed; (+++) strong suppressed in comparison to positive control; (-) not suppressed.
$_{F}$False positive; $_{NA}$Not available

3.2. Flp1, a site-specific recombinase 1 encoded on the 2-micron plasmid is a potential suppressor

Sixteen plasmids that transformants created were able to grow well in the presence of HU were sequenced to identify the genomic DNA fragments inserted. Finally, it revealed fourteen plasmids harboring *SGS1* sequence including either or both of its upstream and downstream sequence and nearby region on chromosome XIII, jointly forming inserted fragment of approximately 6 kb. The presence of *SGS1* in screening results served as a positive control for our suppressor screening approach. The plasmid number 31 contained an upstream sequence (around 250 base pairs) and full length of a gene called *FLP1*, a site-specific recombinase 1 encoded on the 2-micron plasmid which is a multi-copy selfish extrachromosomal DNA element found in the nucleus in budding yeast. Here, it is clear that the construct of genomic DNA fragment found in plasmid number 31 could guarantee the expression of functional Flp1 due to the presence of its native promoter in upstream sequence and completed coding sequence. Next, *FLP1* gene was separately cloned into pRS315 vector and using drop dilution assay, we observed that the Flp1 overexpression could partially rescue the HU sensitivity of the *sgs1Δmus81$_{Δ100N}$* mutant cells (Figure 3). This result confirmed that Flp1 is a single-copy suppressor of the *sgs1Δmus81$_{Δ100N}$* mutant.

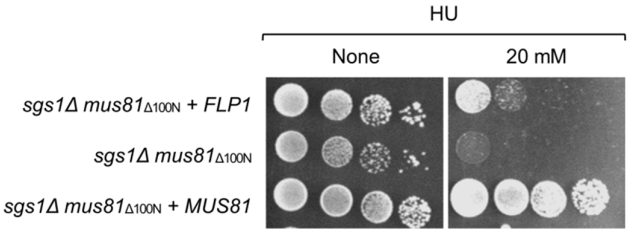

Figure 3. *Overexpression of Flp1 could partially rescue the HU-sensitivity of the sgs1Δmus81$_{Δ100N}$ cells. FLP1* coding sequence was cloned into pRS315 plasmid and its expression was driven by *ADH1* promoter. Empty vector pRS315 or vector containing *MUS81* or *FLP1* was then transformed into the *sgs1Δmus81$_{Δ100N}$* cells.

4. Discussion

Our previous study has shed light on the important role of Mus81 N-terminal region for the full function of Mus81 complex in the absence of Sgs1 during homologous recombination repair pathway [12]. Next, we raised a question for the alternative pathway that can rescue the cellular defect of *mus81* mutant isolated in the study. As described in Results section, we have succeeded to recover the *FLP1* gene on the 2-micron plasmid as the suppressor. The 2-micron circle independently reproduces itself with chromosome-like stability via the joined activity of a plasmid amplification system and a plasmid partitioning system. There has not been obvious evidence for the advantage or disadvantage of this plasmid existence to its host [14]. However, a very high copy numbers

of the plasmid is harmful to the host, leading to cell cycle misregulation and cell lethality [15]. There is a fact that our strain cells should already harbor 2-micron plasmids but were failed to survive when treated with HU without being further transformed with an extra-plasmid containing *FLP1* sequence. This observation can be explained by regulation of the endogenous 2-micron plasmid number and gene expression. Expression of endogenous Flp1 is not relevant to the condition that cell is facing to. However, expression of Flp1 on an extra-plasmid transformed is segregated to the number and regulation of endogenous 2-micron system. The protein is considered as to be overexpressed and can be potentially beneficial when cells are challenged by DNA damaging agents.

We already prove that the lethality of *sgs1Δmus81*$_{\Delta 120N}$ was rescued by further deletion of Rad52, a key homologous recombination mediator in homologous recombination in budding yeast, indicating that the cellular defect related to dysfunction of Mus81 lacking N-terminal region was caused by the accumulation of unprocessed toxic recombination intermediates [12]. Therefore, the suppression of cellular defect of *sgs1Δmus81*$_{\Delta 100N}$ by Flp1 overexpression should be derived from the ability to reduce the recombination intermediates accumulated. At this condition, HU treatment induces quickly high accumulation of toxic intermediates, leading to a condition that the cells may activate Flp1 function in mediating the removal of the toxic intermediates, showing the partially resistant capability to drug.

Until now, the reason of 2-micron plasmid presence inside yeast cell has not been clearly defined, hence, identifying Flp1 as the suppressor of Mus81 partial dysfunction raises the possible explanation of advantage of readily containing this plasmid inside the cells as a backup system. It has not been completely clear that the Flp1 overexpression suppressor effect depends on its enzymatic activities, pointing to an involvement of DNA cleavage and recombination, or its potential function is perhaps merely structural. Therefore, Flp1 should be further investigated for its possible function in resolving homologous recombination intermediates generated when cells try to repair DNA damages.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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