Isolation and Characterization of DNA Damaging Agent Sensitivity of \textit{rqh1} mutant from \textit{Schizosaccharomyce pombe}

In Hye Lee and In Soon Choi*

Department of Biological Science, College of Medical Life Science, Silla University, Busan 617-736, Korea
Received December 5, 2006 / Accepted December 22, 2006

The \textit{Rqh1} gene is essential for vegetative growth in fission Yeast. The \textit{rqh1} mutant showed that sensitivity of DNA damaging agent, a wild range of phenotype including abnormal gene expression and cell elongation. This result showed that the \textit{rqh1}-overexpression cell was sensitivity to DNA damaging agent like \textit{rqh1} mutant. When \textit{Rqh1} have an over-expression by \textit{nrmt1} promoter of pREP vector, \textit{rqh1} mutant DNA damaging agent sensitivity could be compensated. We isolated two strong mutant containing complementation gene, \textit{rqh156} and \textit{rqh172}, respectively. This result observed that the DNA damaging agent sensitivity of \textit{rqh1} mutant was complemented by the expression of \textit{rqh156} and \textit{rqh172}. They induced mRNA expression in a dose-dependent manner HU, MMS and UV. The HU sensitivity of the \textit{rqh1} was complemented by the expression of \textit{rqh156} and \textit{rqh172}. The mRNA expression of \textit{rqh156} decreased on HU dose dependent but the mRNA expression of \textit{rqh172} did not decrease on HU dose dependent. The MMS and UV sensitivity of the \textit{rqh1} was complemented by the expression of \textit{rqh156} and \textit{rqh172}. These results indicate that the isolated \textit{rqh1} gene may play an important role in DNA metabolism.

\textbf{Key words} – RecQ Helicase Family, \textit{rqh1}, Hydroxyurea, Methyl-Methan Sulfonate, Ultraviolet

\textbf{Introduction}

The RecQ helicase family is highly conserved in evolution from prokaryotes to humans and constitutes a sub-family of helicases that contains a characteristic core domain including seven motifs found in many DNA and RNA helicases. Additionally, this domain is also found in certain proteins that are not bona fide helicases, but which utilize ATP hydrolysis to translocate along DNA. This conserved central domain in RecQ helicases are regions that vary both in size and in sequence between the different family members. In eukaryotes, these domains can be quite large, and in the case of WRN an additional catalytic function in the form of a 3’-5’ exonuclease activity is conferred by the extended N-terminal domain. In other cases, these domains have been shown to mediate physical associations with heterologous proteins[3,5,6].

Fission yeast \textit{rqh1} is a member of the RecQ helicase family which includes budding yeast \textit{sgs1} and human \textit{BLM}, \textit{WRN} and \textit{REQL4} helicases which are implicated in Bloom’s, Werner’s and Rothmund-Thomson syndromes, respectively. The RecQ-related family of DNA helicases is required for the maintenance of genomic stability in organisms ranging from bacteria to human. RecQ family helicases possess 3’ to 5’ helicase activity and are required for the maintenance of genomic stability[10,12,13]. The function of RecQ family helicases is always closely linked to Top3 and associated with recombination. In budding yeast, \textit{S. cerevisiae}, \textit{sgs1} deletion mutants also show genomic instability including hyper-recombination of the rDNA locus and a reduced life span that correlates with the accumulation of extrachromosomal rDNA circles. In humans, mutation of three RecQ-related helicases, \textit{BLM}, \textit{WRN} and \textit{REQL4}, cause the cancer-prone and premature ageing diseases of Bloom syndrome (BS), Werner’s syndrome (WS) and Rothmund-Thomson syndrome (RTS), respectively. Cells derived from BS patients show increases in sensitivity to methyl-methane sulfonate, interchange between homologous chromosomes, and sister chromatid exchange (SCE)[8,9,11]. Cells derived from WS patients show chromosome instability, a shorter life span in culture, and accelerated telomere shortening[14,25]. RTS cells show chromosome instability, which becomes manifest as chro-

\*Corresponding author
Tel : +82-51-999-5348, Fax : +82-51-999-5644
E-mail : ischoi@silla.ac.kr
mosome mosaicism and trisomy[4,14,26].

Rqh1 (also called Rad12 and Hus2) is a member of the RecQ subfamily of DNA helicases[12,15,18,27]. A single allele of hus2, hus2-22 (rqh1-h2), was also identified in the screen for hus mutants[17,18,24]. For reasons that will become clear below, we have renamed this gene rqh1. Defects in RecQ helicases typically result in elevated levels of recombination, and problems with DNA replication and chromosome segregation[24,28]. Like the other hus mutants, rqh1 cells undergo an aberrant 'cut'-like mitosis in HU and are also radiation sensitivity. In the case of rqh1 mutants, hyper-recombination is particularly marked following exposure to UV and depletion of deoxynucleotides by HU, which both perturb replication fork progression and result in S-phase arrest. rqh1 cells appear to recover normally from S-phase arrest and continue to complete bulk DNA synthesis[18,20]. S. pombe RecQ-related helicase, causes cells to display reduced viability and elevated levels of chromosome loss. After S-phase arrest or DNA cells lacking rqh1 function display elevated levels of homologous recombination and defective chromosome segregation. In the absence of Rqh1, HJs (Holliday junctions) either remain as a physical link between sister chromatids, preventing their segregation, or are resolved to generate a DSB (double strand break) that is repaired by recombination[1,20,22].

The present study intends to characterize the functional roles of S. pombe Rqh1. The rqh1 mutants are sensitive to DNA damaging agents, how increased levels of 'cut' cells and elevated rates of minichromosome loss following HU treatment. To do this, rqh1 mutants was isolated. The fission yeast, S. pombe, which displays efficient DNA repair systems, was used in this study as a model system for higher eukaryotes. When the Rqh1 have an over-expressed in rqh1 mutant, we tried to find the complementation gene of sensitivity of DNA damage agent. It would be helpful to define the function of Rqh1.

Material and Methods

Strains, cell culture, and genetic methods

E. coli strains were grown on LB media (1% tryptone, 1% sodium chloride, 0.5% yeast extract). Complete (YES) and minimal (SD) growth media were used for growth of S. pombe[1]. Complete and minimal growth media for fission yeast was YES and SD with no leucine. YES was 30 g/L glucose, 5 g/L yeast extract, 0.225 g/L adenine, 0.225 g/L histidine, 0.225 g/L lysine, 0.225 g/L uracil, and 0.225 g/L leucine. Minimal medium was 20 g/L glucose, 6.7 g/L yeast nitrogen base w/o amino acid, 0.225 g/L adenine, 0.225 g/L histidine, 0.225 g/L lysine, and 0.225 g/L uracil. Plasmids were constructed by standard techniques[11] and E. coli DH5α was used as a host for propagation of plasmids. Transformation of S. pombe was performed by the dimethyl sulfoxide (DMSO)-enhanced lithium method[7,22].

The S. pombe (Schizosaccharomyces pomb) and E. coli strains used in this study are listed in Table 1. To make a mutant, pET436 and many strains used (a gift from Tamar Enoch at Harvard Medical Center). The correct transformants were confirmed by Southern blot.

Construction of the rqh1 deletion mutant

To create rqh1 deleted mutants by plasmid shuffling, the 3.6 kb Nhel-AgeI fragment of rqt1B were deleted. This mutant was unable to complement the HU sensitivity of the rqt1-h2 allele. An insert bearing the rqt1 deletion was removed from the pUR19 vector by digestion with SacI and SpII and ligated into SacI and SpII digested pUC19. The 1.7 kb u4A gene was then inserted into the NsiI digested rqt1 deletion construct as described previously[11,20]. This plasmid (pTE436, see Table 1: Strains provided by T. Enoch and T. Cech) was digested with SacI and SpII and the linear disruption construct, consisting of the u4A gene flanked by rqt1 sequences, was isolated. This DNA was used to disrupt one copy of the rqt1 gene by the one-step disruption method in an h'/h u4A-D18/ u4A-D18 stable.

Table 1. Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF199</td>
<td>hiu1-32 his-3-D1 u4A-D18 ade6-M210 (wild type)</td>
</tr>
<tr>
<td>1389</td>
<td>hiu1-32 his-3-D1 u4A-D18 rqt1:u4A*</td>
</tr>
<tr>
<td>TE480</td>
<td>diploid ade-M216/ade-M216 leu1-32/leu1-32 u4A-D18/ u4A-D18 h'/h</td>
</tr>
<tr>
<td>TE558</td>
<td>diploid ade6-M210/ade6-M210 leu1-32/leu1-32 u4A-D18/ u4A-D18 h'</td>
</tr>
<tr>
<td>TE786</td>
<td>rqt1::u4A* u4A-D18 ade6-M210 containing Ch</td>
</tr>
<tr>
<td>TE788</td>
<td>ade6-M210 containing Ch</td>
</tr>
<tr>
<td>TE767</td>
<td>rqt1::u4A* u4A-D18 h</td>
</tr>
</tbody>
</table>

Plasmid | Constructions |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pTE151</td>
<td>rqt1B</td>
</tr>
<tr>
<td>pTE152</td>
<td>rqt1A</td>
</tr>
<tr>
<td>pTE436</td>
<td>rqt1 disruption construct</td>
</tr>
</tbody>
</table>
diploid (TE480, see Table 1; a generous gift from G. Cottarel and MS O). The homologous integration event was confirmed by Southern blotting. This strain was crossed to an het/letura4-D18/letura4-D18 stable diploid (TE588, see Table 1) to generate a sporulating diploid heterozygous for the rph1 deletion. These diploids were sporulated, tetrads were dissected and haploids that were HU sensitive and ura1 were identified. The HU-sensitive and ura1 phenotypes were found to co-segregate and to segregate 2:2 in all of the tetrads analyzed. One such haploid was picked for further analysis and southern blot analysis was used to confirm deletion of the rph1+ gene (TE767, see Table 1). Further crosses established that rph1Δ was allelic to rph1-h2.

The preparation of Rqh1 overexpression strains

The pREP41-HAN vector was used for Rqh1 overexpression. Cells bearing rph1-overproducing plasmid were first grown in EMM medium containing 4 mM thiamine (transcription repressed), washed three times with EMM medium, and further grown in EMM lacking thiamine for 20 hour (transcription induced at 14-16 hour). The overexpression of HA-Rqh1 was confirmed by western blot using anti-HA antibody (Boehringer Mannheim).

Sensitivity test to hydroxyurea (HU), Methyl-Methan sulfonate (MMS) and ultraviolet (UV)

Cells were grown to mid-log phage, washed with fresh SD medium, and then resuspended at a concentration of 1 × 10^7 cell/mL. In duplicate, serial 10-fold dilutions were spotted onto SD agar plates containing the damage agent and onto a control plate (none damage agent). HU plates contained 4 mM HU. MMS plates contained 0.004 % MMS. UV plates exposed 50 J/m² UV. As before, each plate was incubated at 30°C for 5 days.

Three days cultures of wild type CF199 and 1589 mutants, 1589#56, 1589#72 growing at 30°C were diluted to 1 × 10^6 cell/mL. Appropriate dilutions containing 1000 cells were plated onto SD agar plates contained HU, MMS and onto SD agar plates exposed UV which were incubated at 30°C for 5 days, and then numbers of the colonies were counted.

Results and Discussion

Identification of rhf1 (1589) mutant

Goodwin et al. [12] examined the phenotypic con-

sequences of germinating top3 deleted spores and observed abnormal nuclei, multiple septa and elongated cells. However, they were not able to directly examine the role of Top3 in DNA damage repair or chromosome stability because the only viable top3 defective cells examined were, by necessity, also deleted for the rph1 gene. In our study, we have examined the phenotype of top3 deficient cells by creating top3-ts mutants. Following a temperature shift to the restrictive temperature, we see morphological pheno-

types that are very similar to those reported by Goodwin et al.[12]. These above results indicate that Rqh1 might play a role in DNA replication step to exert to propagate accurate DNA structures and information to daughter cells. Rqh1 gene was constructed using Dr. MS O’s method[20] (Fig. 1). However, their exact roles needed to elucidate in detail. For these purpose, we tried to search the suppressor gene. To explore the cellular functions of rph1 mutant, the recepient yeast host strain was constructed. The rph1::ura4' diploid strain was transformed with rph1Δ plasmid and sporulated. The plasmid containing rph1 deletion allele by in vitro mutageneses was inserted into host strain and the

![Fig. 1. Schematic representation of RecQ helicases found in various organisms.](image-url)
wild type gene was removed by 5-FOA treatments. The \( nph1 \) deletion mutant alleles were identified by comparison for growth at 30°C. Which its over-expression could compensate the DNA damage sensitivity of \( nph11589 \) mutant, which their HU, MMS and UV sensitivity could be diminished, when they have an over-expressed protein by \( nmt1^+ \) promoter of pREP vector[20] (Fig. 2). To select mutant containing complementation gene, the S. pombe cDNA library with \( nmt1^+ \) promoter of pREP vector was transformed into the \( nph11589 \) mutant. the transformants were then selected at 30°C incubation. Approximately 600 transformants were spotted and screened on selective media for the complementation of DNA damage agent sensitivity (Fig. 3). We identified two potentially mutant containing complementation gene by this procedure. These isolated mutant were renamed, \( nph156 \), \( nph172 \) mutant cell that DNA damaging sensitivity of \( nph11589 \) could be complemented. The complementation of sensitivity in HU, MMS and UV were compared in a \( nph11589 \) and a \( nph156 \), \( nph172 \). All they were viable to control plate (Fig. 3). However \( nph11589 \) mutant could not grow to plates treated HU, MMS and UV, but similar to control sensitivity, the \( nph156 \) and \( nph172 \) were able to grow to HU, MMS and UV at 30°C (Fig. 3C, 3D, 3E) result indicates that the expression of \( nph156 \) and \( nph172 \) functionally complemented the sensitivity of \( nph11589 \) mutant DNA damat.

A viability identification of the cell after the addition of DNA damage agent

We found a mutants survival rate regarding DNA damage agents (HU, MMS, UV). As shown in Fig. 4, it indicated survival rate of wild type (CF199), \( nph11589 \) mutant, \( nph156 \) and \( nph172 \) in HU in a dose-dependent manner from 0 to 4 mM HU. The survival of wild type maintained from 0 mM HU to 4 mM HU. The survival of \( nph11589 \) mutant decreased rapidly on HU dose dependent and there is hardly survival rate on 4 mM HU. On the other hand, survival of \( nph156 \) and \( nph172 \) maintained like a wild type. The survival of wild type maintained from 0 % MMS to 0.004 % MMS. The survival of \( nph11589 \) mutant decreased rapidly to 0.002 % MMS dose dependent and there is hardly survival rate since (Fig. 4). On the other hand,
Fig. 4. The relative to survival of rqh1 mutant cell. The wild-type and mutant cells were incubated onto SD-Leu+ plates treated with HU (A), MMS (B) and UV (C). Each plate incubated at 30°C for 4 days.

Survival of rqh156 and rqh172 were lower than a wild type but were complemented more than rqh11589 mutant. As shown in Fig. 4C, it indicated survival rate of wild type, rqh11589 mutant, rqh156 and rqh172 in UV in a dose-dependent manner from 0 to 50 J/m² UV. The survival of wild type maintained from 0 J/m² UV to 50 J/m² UV. The survival of rqh11589 mutant decreased rapidly to 30 J/m² UV dose dependent and there is hardly survival rate. On the other hand, survival of rqh156 and rqh172 maintained like a wild type.

These results indicated that the rqh156 and rqh172 are to have the survival rate such as wild type unlike rqh11589 mutant about DNA damage agent. Therefore we are regarded that they are complemented according DNA damage agent.

Acknowledgements

The authors thank to Dr. Mi-Sook O for providing rqh1-related genes and invaluable comments. We thank Drs T. Enoch, T. Cech, R. Allshire and R. Gwilliam for providing yeast strains and plasmids (described Table 1).

References

USA 94, 3860-3865.


초록: 분열형 효모인 *Schizosaccharomyces pombe*로부터 rqh1 돌연변이의 DNA damaging agent sensitivity를 보상하는 유전자 특성 연구

이인혜·최인순*  
(신라대학교 생명과학과)

분열형 효모에서 Rqh1은 Top3과 함께 vegetative growth에 필수적이다. *rqi1* 돌연변이는 DNA damaging agent에 민감성을 보이며, 이 때, 부적절한 유전자 발현, 세포 신장, 염색체의 불안정성, 비정상적인 다중격막 발아의 결함을 포함한 넓은 범위의 표현형을 보인다. *rqi1*-overexpression cell 역시 *rqi1* deletion mutant에서 보이는 DNA damaging agent 민감성을 관찰할 수 있다. *rqi1*-overexpression cell의 경우 *rqi1* deletion mutant에서 보이는 DNA damaging agent 민감성을 관찰할 수 있다. *rqi1*-overexpression cell의 경우 *rqi1* deletion mutant에서 보이는 DNA damaging agent 민감성을 관찰할 수 있다. *rqi1* deletion mutant의 DNA damaging agent 민감성은 *rqi156*, *rqi172* 두 개의 돌연변이를 나타내였다. *rqi1* deletion mutant의 DNA damaging agent 민감성은 *rqi156*, *rqi172*의 발현에 의해 보상 되어지는 것을 확인하였다.