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# DNA repair activity of human DNA glycosylases OGG1, NEIL1, NEIL2, and NEIL3 in a fission yeast strain lacking catalase and DNA glycosylase Nth1p activities

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**Human cells have five DNA glycosylases OGG1, NTHL1, NEIL1, NEIL2, and NEIL3 with overlapping substrate ranges to repair damage to oxidative bases. To investigate the DNA repair activity of each enzyme *in vivo*, we expressed OGG1 and three NEIL-family proteins in a fission yeast *Schzosaccharomyces pombe* lacking catalase and DNA glycosylase Nth1p activities. *S. pombe* Nth1p is a unique enzyme that excises oxidatively modified bases in the yeast cells, and a defect of the catalase gene (*ctt1*) enhances the sensitivity of *nth1*Δ cells to $H_2O_2$. Complementation of the yeast $H_2O_2$-sensitivity by OGG1 and NEIL-family proteins showed that all enzymes efficiently initiated base excision repair to correct $H_2O_2$-damaged DNA *in vivo*. The *nth1*Δ/*ctt1*Δ cells were also sensitive to an alkylating agent methyl methanesulfonate (MMS) by accumulating AP sites. Based on the ability to complement MMS sensitivity, human DNA glycosylases were shown to act on AP sites more weakly than *S. pombe* Nth1p. Moreover, we showed that some NEIL3 mutants could complement the DNA repair activity of *nth1*Δ/*ctt1*Δ cells *in vivo*. The results of this study also indicate that *S. pombe ctt1*Δ/*nth1*Δ cells provide a useful heterologous expression system to investigate the *in vivo* function of DNA glycosylase.**

**Keywords**: base excision repair; catalase; DNA glycosylase; NEIL; OGG1; *Schizosaccharomyces pombe.*

## 1. Introduction

DNA molecules in aerobic organisms suffer damage due to reactive oxygen species (ROS), which are constantly generated as by-products of normal metabolism and by exposure to UV-A, ionizing radiation or chemical mutagens (Lindahl, 1993; Friedberg *et al.*, 2006). Oxidation of DNA by ROS generates base damage, apurinic/apyrimidinic (AP) sites, and strand breaks, which result in various biological consequences including mutagenesis, carcinogenesis and aging (Cooke *et al.*, 2003; Friedberg *et al.*, 2006).

Base excision repair (BER) is the major pathway for repair of damage to oxidative bases in DNA, which begins by removal of the altered bases through the use of damage-specific DNA glycosylases (Ide & Kotera, 2004; Friedberg *et al.*, 2006; Hedge *et al*, 2008). In human cells five DNA glycosylases with AP lyase activity (bifunctional glycosylases) have been found to participate in BER of oxidative DNA damage. A DNA glycosylase OGG1 excises 8-oxoguanine (8-oxoG) paired with cytosine, which is the most common lesion generated by the oxidation of guanine. Mispair of 8-oxoG with adenine results in a G:C to T:A mutation. Thymine glycol (Tg), the oxidation product of thymine, interrupts DNA replication and transcription. A human homolog of *Eshcherichia coli* endonuclease III (NTHL1) excises Tg and oxidized pyrimidines from DNA. Both OGG1 and NTHL1 also remove ring-opened oxidative products of purine (formamidopyrimidine derivatives, Fapy). In 2002, three new human DNA glycosylases (NEIL1, NEIL2, and NEIL3), which share homology with *E. coli* endonuclease VIII (Nei), were discovered

(Hazra *et al.*, 2002; Morland *et al.*, 2002; Takao *et al.*, 2002). NEIL1 catalyzes the repair of Fapy, Tg and 5-hydroxyuracil, whereas NEIL2 acts on certain oxidized products of pyrimidines. Recently, a mouse NEIL3 was shown to remove a broad spectrum of oxidative bases on single-stranded DNA with preference for hydantoins, which are further oxidation products of 8-oxoG (Takao *et al.*, 2009; Liu *et al.*, 2010).

Knockout mice lacking OGG1, NTHL1, NEIL1, NEIL2, or NEIL3 are viable and healthy into adulthood (Tsuzuki *et al.*, 2007; Vartanian *et al.*, 2006; Toris *et al.*, 2005). Multiple DNA glycosylases in human cells could provide back-up functions for repair of oxidative DNA damage because of their overlapping substrate range. Thus, the role of these enzymes *in vivo* remains unclear. In the present study, we heterologously expressed human DNA glycosylases in a mutant strain of fission yeast lacking its endonuclease III homolog (Nth1p) and catalase activities. Because of the absence of OGG1 and endonuclease VIII homologs in *S. pombe*, Nth1p is the sole bifunctional DNA glycosylase responsible for removal of oxidatively modified bases (Osman *et al.*, 2003; Sugimoto *et al.*, 2005). Deletion of the catalase gene (*cct1*) enhances the sensitivity to $H_2O_2$ treatment (Mutoh *et al.*, 1999; Hida & Ikeda, 2008).

In this study, we showed that expression of OGG1, NEIL1, NEIL2, and NEIL3 restored the resistance to $H_2O_2$ of the *ctt1*Δ/*nth1*Δ strain. The result indicates that each enzyme individually exhibits enough activity for repairing oxidative DNA damage *in vivo*. Using this heterologous expression system we also attempted to characterize the putative catalytic domain and residue of NEIL3.

## 2. Materials and Methods

### 2.1. Strains and media

*S. pombe* strain ED0665 (ATCC no. 96993; *h*⁻, *ade*6-M210, *leu*1-32, *ura*4-D18) was used as the wild-type strain. The strain *ctt1*Δ/*nth1*Δ (*h*⁻ *leu*1-32 *ura*4-D18 *ctt1*::*kanMX6* *nth1*::*ura4*⁺) is a double disruptant of *ctt1* and *nth1* genes (Hida & Ikeda, 2008). Yeast cells were routinely grown on YE medium (0.5% yeast extract and 3% glucose).

### 2.2. Expression of human DNA glycosylases in yeast cells

pAUR224 (Takara Bio Inc., Otsu, Shiga, Japan) was used as an expression vector in *S. pombe*. cDNAs of human DNA glycosylase genes *OGG1*, *NEIL1*, *NEIL2*, and *NEIL3* were gifts from Dr. Tapas K. Hazra (University of Texas Medical Branch, Galveston, Texas, USA). The sequences were amplified by PCR using KOD DNA polymerase (Toyobo Co., Ltd., Osaka, Japan) and specific primers shown in Table 1. The forward and reverse primers contain a tag sequence of *Sal*I and *Bcl*I restriction sites, respectively, at their 5′ ends. The amplified DNA fragments were cloned into pAUR224 using the *Sal*I and *Bam*HI sites. The OGG1, NEIL1, NEIL2, and NEIL3 expression plasmids and *S. pombe* Nth1p expression plasmid (Sugimoto *et al.*, 2005) were introduced to yeast cells using the *S. pombe* Direct Transformation Kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Transformants were selected on YE medium containing 0.3 μg/ml aureobasidin A (Takara Bio Inc.). Mutagenesis of NEIL3 was performed by inverse PCR using the KOD-Plus-Mutagenesis Kit (Toyobo Co., Ltd.) and appropriate primer sets shown in Table 1.

### 2.3. Survival assay

Cells were grown overnight in YE medium for survival studies. Cell density was determined by a

Table 1. PCR primers used in this study.

| Primer | Sequence (5′ to 3′) | Use |
|---|---|---|
| hOGG1(+1)SalI-Fw | ATTAGTCGACATGCCTGCCCGCG | Amplification of *OGG1* cDNA |
| hOGG1(+1035)BclI-Rv | ATTTGATCAGCCTTCCGGCCCTTTG | |
| hNEIL1(+1)SalI-Fw | ATTAGTCGACATGCCTGAGGGCCCC | Amplification of *NEIL1* cDNA |
| hNEIL1(+1173)BclI-Rv | GCGTGATCACTAAGAGGCTGAGGTCC | |
| hNEIL2(+1)SalI-Fw | ATATGTCGACATGCCAGAAGGGCCG | Amplification of *NEIL2* cDNA |
| hNEIL2(+999)BclI-Rv | TCCGTGATCATTAGGAGAACTGGCACTG | |
| hNEIL3(+1)SalI-Fw | ATATGTCGACATGGTGGAAGGACCAG | Amplification of *NEIL3* cDNA |
| hNEIL3(+1818)Bcl-Rv | GGCGCTGATCAGCATCCAGGAATAAT | |
| NEIL3GD(+1816)-Fw | TAAGGATCCACCGGATCTAGATAA | Preparation of NEIL3-GD |
| NEIL3GD(+870)-Rv | GCATATGTCAACATGTTGAGGATTTTC | |
| NEIL3-m3P-Fw | CCGGAAGGACCAGGCTGTACTCTGAAT | Preparation of NEIL3-V2P |
| NEIL3-m1-Rv | CATCTCGACTGCAGAATTCGAAGCTTGAGC | |

hemocytometer, and adjusted to $1.0 \times 10^7$ cells/ml with YE medium. The cells were serially diluted 10-fold from $10^{-1}$ to $10^{-3}$ in YE medium. To measure the sensitivity of cells to chronic exposure to DNA damaging agents, 10 μl of dilutents containing $1 \times 10^2$ to $1 \times 10^5$ cells were spotted onto YE medium containing $H_2O_2$ (0 to 0.9 mM) or MMS (0 to 0.025%). Photographs of colonies were taken after 3 days of growth at 28°C.

### 3. Results and Discussion

Human cells have five DNA glycosylases with overlapping substrate specificities to repair damage to oxidative bases. To evaluate the DNA repair activity of these individual enzymes *in vivo*, effects of back-up function by redundant enzymes should be eliminated from the cells. Conveniently, *S. pombe* has a unique enzyme Nth1p that excises oxidatively modified bases in its cells. Although Nth1p contributes *in vivo* to avoid ROS-induced mutagenesis and cell death, catalase (Ctt1p) activity obscures the functions of Nth1p in wild-type cells (Hida & Ikeda, 2008). Therefore, we expressed human DNA glycosylases in *S. pombe ctt1Δ/nth1Δ* cells, and measured the survival rate of the yeast cells after chronic exposure to oxidative stress (Fig. 1A). *S. pombe ctt1Δ/nth1Δ* cells exhibited severe sensitivity to $H_2O_2$. Expression of *S. pombe* Nth1, as a control, restored resistance to $H_2O_2$ near to the level of wild-type cells. Similarly, expression of NEIL1,

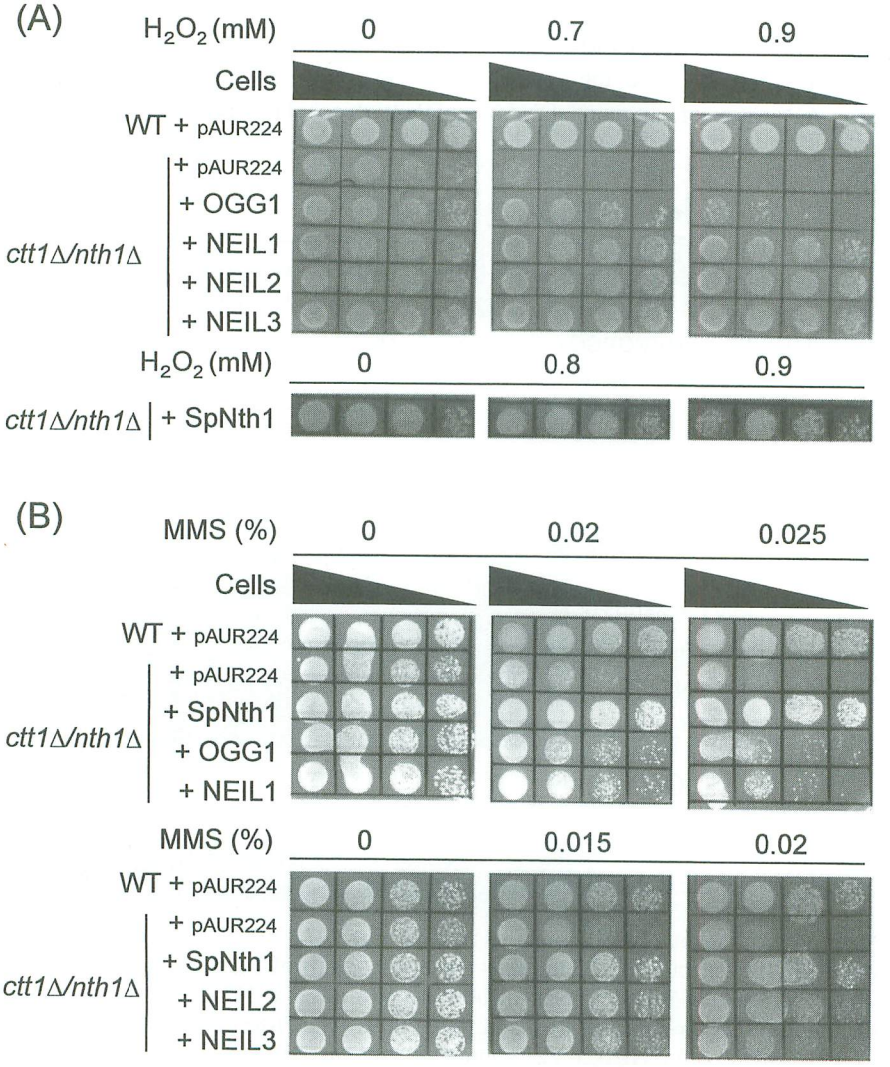


Fig. 1. DNA repair activity of human DNA glycosylases in *S. pombe ctt1Δ/nth1Δ* cells. The *ctt1Δ/nth1Δ* cells harboring a plasmid expressing the human DNA glycosylases (OGG1, NEIL1, NEIL2, and NEIL3) and *S. pombe* Nth1p (SpNth1) were grown overnight in YE medium and survival assays for $H_2O_2$ (A) and MMS (B) were carried out as described in the Materials and Methods. Wild-type (WT) and *ctt1Δ/nth1Δ* cells harboring the empty vector pAUR224 were used as controls.

NEIL2, and NEIL3 restored full resistance. On the other hand, OGG1 exhibited moderate sensitivity to $H_2O_2$. The results indicate that each NEIL-family glycosylase initiates a BER pathway to repair $H_2O_2$-damaged DNA *in vivo* with the same efficiency as *S. pombe* Nth1p. OGG1 partially complements the DNA-repair defect of *ctt1Δ/nth1Δ* cells, possibly due to its narrow substrate range.

In *S. pombe* cells, AP sites are generated by monofunctional DNA glycosylases such as Mag1p for alkylated bases, and are mainly processed by AP lyase activity of Nth1p to leave 3′-α,β-unsaturated aldehyde ends (Nth1p-dependent BER) (Tanihigashi *et al.*, 2006: Kanamitsu & Ikeda, 2010). The 3′-blocked end must be converted to 3′-OH by Apn2p. Thus, *nth1Δ* cells are sensitive to alkylating agents such as methyl methanesulfonate (MMS) by accumulation of AP sites. To evaluate the repair activity toward the AP site of human DNA glycosylases *in vivo*, we expressed these enzymes in *S. pombe ctt1Δ/nth1Δ* cells, and measured the survival rate of the yeast cells after chronic exposure to MMS (Fig. 1B). As expected, *S. pombe ctt1Δ/nth1Δ* cells exhibited sensitivity to MMS. Expression of *S. pombe* Nth1 in *ctt1Δ/nth1Δ* cells restored the resistance to MMS near the level of wild-type cells. On the other hand, expression of OGG1, NEIL1, NEIL2, and NEIL3 partially complemented the resistance to MMS. The results indicate that human DNA glycosylases could process AP sites through their AP lyase activity to repair the lesion through the BER pathway. OGG1 cleaves the AP site via β-elimination, and NEIL1, NEIL2, and NEIL3 cleave via β,δ-elimination (Hegde *et al.*, 2008; Liu *et al.*, 2010). A direct comparison of the kinetic constant of the human enzymes with the AP site-containing DNA has not yet been made. Based on the ability of complementation *in vivo*, the human DNA glycosylases are shown to act on AP sites more weakly than *S. pombe* Nth1p.

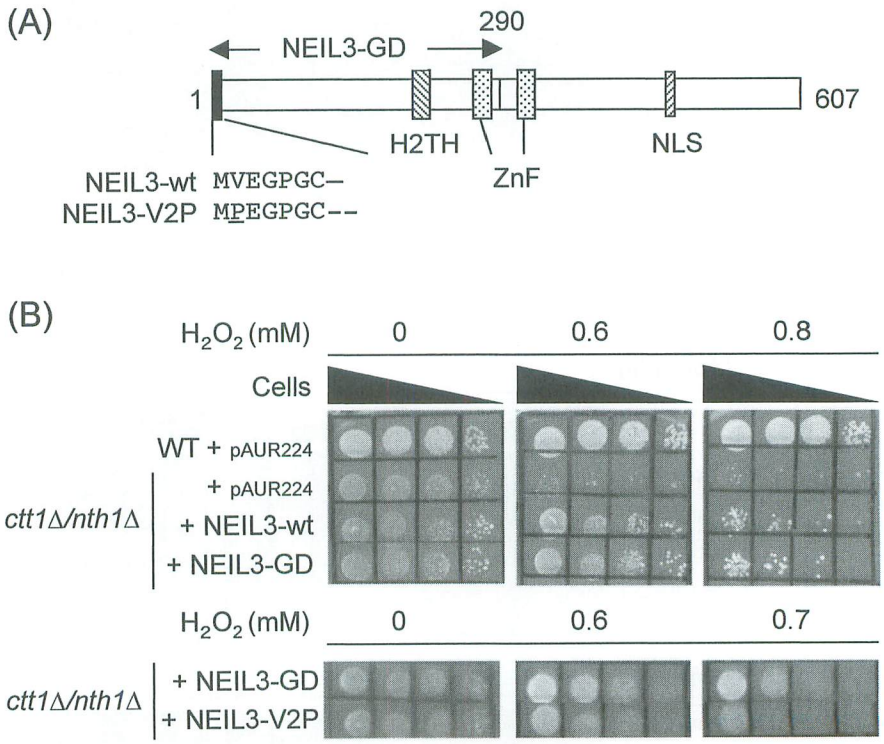


Fig. 2. DNA repair activity of various NEIL3 mutants in *S. pombe ctt1Δ/nth1Δ* cells. (A) Domain structure of human NEIL3 protein and the various NEIL3 mutants. N-terminal half of NEIL3 is composed of a Nei-like glycosylase domain (NEIL3-GD). Putative motifs of helix-two-turn-helix (H2TH), zinc finger (ZnF), and nuclear localization signal (NLS) are marked (Takao *et al.*, 2009). The N-terminal amino acid sequences of wild-type NEIL3 (NEIL3-wt) and the NEIL3-V2P mutant are shown. (B) The *ctt1Δ/nth1Δ* cells harboring a plasmid expressing wild-type NEIL3 (NEIL3-wt) and its mutants (NEIL3-GD and -V2P) were grown overnight in YE medium and survival assays for $H_2O_2$ were carried out as described in the Materials and Methods. Wild-type (WT) and *ctt1Δ/nth1Δ* cells harboring the empty vector pAUR224 were used as controls.

Among the human Nei homologs, the function of NEIL1 and NEIL2 in the BER pathway has been extensively studied, but the enzymatic properties of NEIL3 remain unclear. As shown in Fig. 2A, NEIL3 is composed of two major domains, a Nei-like glycosylase domain (NEIL3-GD) at the N-terminal half and a C-terminal domain (Takao *et al.*, 2009; Liu *et al.*, 2010). Putative motifs of helix-two-turn-helix (H2TH), zinc finger (ZnF), and a nuclear localization signal (NLS) are present. To test the catalytic activity of the NEIL3-GD domain in the repair of oxidative DNA damage *in vivo*, we expressed the domain in *S. pombe ctt1Δ/nth1Δ* cells, and measured the survival rate of the yeast cells after chronic exposure to $H_2O_2$ (Fig. 2B). NEIL3-GD restored the resistance of the yeast mutant to $H_2O_2$ at the same level of full-length NEIL3. In agreement with this result, Takao *et al.* (2009) also showed that NEIL3-GD cleaved a single-stranded oligonucleotide containing an AP site faster than full-length NEIL3. These results indicate that the C-terminal half of NEIL-3 is dispensable for catalysis, and possibly constructs the domain for regulating the enzyme activity of or interaction with other proteins *in vivo*.

The Nei homologs of several organisms have a well-conserved proline residue next to their N-termini, which serves as a nucleophile of the β,δ-elimination reaction (Burgess *et al.*, 2002). The third glutamic acid residue is also strictly conserved among Nei-family proteins. However, the second amino acid of human NEIL3 is valine (Fig. 2A). We examined the importance of the valine residue for catalysis of NEIL3 in DNA repair activity using *S. pombe ctt1Δ/nth1Δ* cells (Fig. 2B). A mutant NEIL3 harboring a valine-to-proline substitution (NEIL3-V2P) exhibited moderate resistance to $H_2O_2$, suggesting that proline could function as a catalytic residue of NEIL3 *in vivo*. Takao *et al.* (2009) also showed that NEIL3-V2P, -V2T, and -E3A mutants have the same activity as wild-type NEIL3, and that NEIL3-NΔ3 completely lost AP lyase activity *in vitro*.

In conclusion, we expressed the human DNA glycosylases OGG1, NTHL1, NEIL1, NEIL2, and NEIL3 in *S. pombe ctt1Δ/nth1Δ* cells, and showed that each enzyme exhibits redundant activity in the repair of oxidative damage and the AP site. The results further show that *S. pombe ctt1Δ/nth1Δ* cells are useful for investigating the *in vivo* function of DNA glycosylase.

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