Overexpression of Bacterioferritin Comigratory Protein (Bcp) Enhances Viability and Reduced Glutathione Level in the Fission Yeast Under Stress

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The structural gene encoding bacterioferritin comigratory protein (Bcp) was amplified using PCR from the genomic DNA of *Schizosaccharomyces pombe*, and transferred into the shuttle vector pRS316 to generate the recombinant plasmid pBCP10. The bcp^+ mRNA level in the pBCP10-containing yeast cells was significantly higher than that in the control yeast cells, indicating that the cloned gene is functioning. The *S. pombe* cells harboring the plasmid pBCP10 exhibited higher survival on the solid minimal media with hydrogen peroxide, *tert*-BOOH or cadmium than the control yeast cells. They also exhibited enhanced cellular viability in the liquid media containing the stressful agents. The increased viabilities of the fission yeast cells harboring the plasmid pBCP10 were also obtained with 0.4% glucose or 0.4% sucrose as a sole carbon source, and nitrogen starvation, compared with those of the control yeast cells. The total glutathione (GSH) content and total GSH/GSSG ratio were significantly higher in the yeast cells harboring the plasmid pBCP10 than in the control yeast cells. In brief, the *S. pombe* Bcp plays a protective role in the defensive response to oxidative stress possibly via up-regulation of total and reduced glutathione levels.

Keywords: fission yeast, bacterioferritin comigratory protein, glutathione, peroxiredoxin, *Schizosaccharomyces pombe*, stress

Peroxiredoxins (Prxs), also known as thioredoxin peroxidases, are a family of antioxidant proteins ubiquitously conserved in a wide variety of living organisms, and don't share any sequence homology with other antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, and catalase (Bast *et al.*, 2002). They play an important role in the cellular defense against oxidative and nitrosative stress through their peroxidase and peroxynitrite reductase activities supported by thioredoxin, cyclophilin, and glutaredoxin (Lee *et al.*, 2001; Wong *et al.*, 2002). Prxs, produced at high levels in living cells, are located primarily in cytosol, and also identified within mitochondria, chloroplasts, peroxisomes, nuclei, and membranes (Hofmann *et al.*, 2002). Although all Prx classes share the same catalytic mechanism, in which an active site cysteine (the peroxidase cysteine) is oxidized to a sulfenic acid by the peroxide substrate, they undertake the different recycling of the sulfenic acid to a thiol (Wood *et al.*, 2003).

Escherichia coli bacterioferritin comigratory protein (Bcp), a member of the thiol-specific antioxidant (TSA)/alkyl hydroperoxide peroxidase C (AhpC) family, is classified into a group of Prxs since Bcp contains thioredoxin-dependent hydroperoxide peroxidase activity (Jeong *et al.*, 2000). The TSA/AhpC family has a conserved cysteine as the primary site of catalysis instead of the selenocysteine of glutathione peroxidase (Jeong *et al.*, 2000). A Bcp homologue of the plant *Sedum lineare* also revealed thioredoxin-dependent peroxidase activity, the hallmark of Prx family, and two cysteine residues are well conserved among the Bcp family (Kong *et al.*, 2000). In the thioredoxin-dependent manner, poplar peroxiredoxin Q, as one of the four plant subtypes and a homologue of Bcp, reduces various alkyl hydroperoxides, but with a better efficiency for cumene hydroperoxide than hydrogen peroxide and *tert*-BOOH (Rouhier *et al.*, 2004).

In the fission yeast *S. pombe*, Tpx1, a 2-Cys Prx, is required for peroxide-induced oxidation and nuclear accumulation of AP1-like transcription factor Pap1, and reduced by a sulphiredoxin, Srx1, which allows rapid activation of Pap1 at high concentrations of hydrogen peroxide (Bozonet *et al*., 2005). The *S. pombe* Tpx1 is also required for peroxide-induced activation of Sty1, a p38/JNK homologue, with the formation of a peroxide-induced disulfide complex between Tpx1 and Sty1 (Veal *et al.*, 2004). The *S. pombe* Prx, a second Prx identified in the fission yeast, is linked with the yeast growth and up-regulated by metabolic oxidative stress on a transcriptional level (Kang *et al.*, 2008). The Prx protein is partly responsible for maintaining low ROS level under normal and stressful growth conditions in the fission yeast (Kang *et al.*, 2008). In this work, we demonstrate that a Bcp homologue is involved in viabilities and up-regulation of reduced glutathione level in the fission yeast *S. pombe* under normal and/or stressful conditions.

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Materials and Methods

Chemicals

Ampicillin, bovine serum albumin (BSA), L-leucine, uracil, adenine, glucose, glutathione (GSH), glutathione reductase, NADPH, 2-vinylpyridine, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and Bradford reagent were purchased from Sigma Chemical Co. (USA). Restriction enzymes (*Eco*RI and *Xho*I), T4 DNA ligase, RNaseA, and Ex *Taq* polymerase were obtained from TaKaRa Shuzo Co. (Japan). Yeast extract and agar were obtained from Amersham Life Science (USA). TRIZOL® Reagent and 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) were from Invitrogen (USA). M-MLV reverse transcriptase was from Promega (USA). PCR primers were from COSMO Co. (Korea). All other chemicals used were of highest grade commercially available.

Strains and growth conditions

E. coli MV1184 [*ara* Δ*(lac-proAB) rpsL thi* (*Φ80 lacZΔM15*) Δ(*sr1-recA*)*306::*Tn*10(tet^r)* / F' (*traD36 proAB$^+$ lacI^q lacZΔM15*)] and *S. pombe* KP1 (*h$^+$ leu1-32 ura4-294*) were used. The yeast cells were grown in minimal medium, which contained KH phthalate (3 g), Na_2HPO_4 (1.8 g), NH_4Cl (5 g), D-glucose (20 g), 1,000× vitamin mixture (1 ml), 10,000× minerals (0.1 ml), 50× salts (20 ml), and L-leucine (250 mg) per 1 L. Salts stock (50×) contains 5.2 mM $MgCl_2·6H_2O$, 0.1 mM $CaCl_2·2H_2O$, 13.4 mM KCl, and 0.28 mM Na_2SO_4. Minerals stock (10,000×) contains 8.1 μM H_3BO_3, 2.37 μM $MnSO_4$, 1.39 μM $ZnSO_4·7H_2O$, 0.74 μM $FeCl_3·6H_2O$, 0.25 μM $MoO_4·2H_2O$, 0.6 μM KI, 0.16 μM $CuSO_4·5H_2O$, and 4.76 μM citric acid. Vitamins stock (1,000×) contains 81.2 μM nicotinic acid, 55.5 μM inositol, 40.8 μM biotin, and 4.2 μM pantothenic acid. The yeast cultures were shaken at 30°C, and the cell growth was monitored by the absorbance at 600 nm.

Plasmid

E. coli-yeast shuttle vector pRS316 (Myers *et al*., 1986) was used for cloning the full-length genomic DNA of the *bcp$^+$* gene from *S. pombe*.

Cell harvesting and preparation of cellular extracts

The appropriate number of the yeast cells was harvested by centrifugation. They were resuspended in 20 mM Tris buffer (pH 8.0) – 2 mM EDTA and disrupted using a glass bead beater. Supernatant obtained after centrifugation was used as the crude extract for the GSH assay detailed below.

Cloning

Based on the nucleotide sequence stored in the GenBank database (CAA21907), the genomic DNA encoding the *S. pombe* Bcp was amplified by PCR. PCR was performed according to the manufacturer's recommended protocol. The PCR condition used in amplifying the *bcp$^+$* gene was 94°C (denaturation, 1 min), 55°C (annealing, 1 min), and 72°C (extension, 1 min) for 30 cycles with the two primers (primer 1, 5'-ttgcatgcaaatgaattcctagctgatgg-3'; primer 2, 5'-atttgaatgt atctcgagggttgaagcac-3') containing *Eco*RI and *Xho*I sites, respectively. The digested PCR product was ligated into pRS316 previously digested with *Eco*RI and *Xho*I, and the ligation mixture was used to transform the *E. coli* strain MV1184. The resultant recombinant plasmid pBCP10 was confirmed by restriction mapping and nucleotide sequencing.

Nucleotide sequencing

Nucleotide sequencing was performed by COSMO Co. using an automatic DNA sequencer. The determined sequence of the *S. pombe bcp$^+$* gene reported in this article has been submitted to the GenBank database under the accession number EU266495.

Protein determination

Protein content in extracts was determined according to the procedure of Bradford (1976) using bovine serum albumin as a standard.

RT-PCR analysis

Total RNA was prepared using TRIZOL® reagent according to manufacturer's protocol. First-strand cDNA was synthesized from 2 μg total RNA using M-MLV reverse transcriptase. One twentieth of the synthesized first-strand cDNA was used as templates in PCR. The two synthetic primers used were primer 3; 5'-gattgcaaacgtcttggatagcaaa-3' and primer 4; 5'-gcaaaggctttgtcaacactgacaa-3'. PCR was performed using Ex *Taq* polymerase as follows: denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min for 30 cycles.

Determination of total glutathione

Total glutathione in extract was determined as previously described (Floreani *et al.*, 1997). The reaction mixture (200 μl) contained 160 μl of 0.1 M phosphate buffer (pH 7.4)~10 mM EDTA, 10 μl of 4.8 mM NADPH, 5 μl of 4 mM DTNB and 20 μl of cellular extract. After 3 min equilibration at 25°C, the reaction was started by adding 5 μl of 6 units/ml glutathione reductase, and the formation of 2-nitro-5-thiobenzoic acid was recorded at 412 nm.

Determination of GSSG

Oxidized glutathione (GSSG) in extract was determined according to a modification of the previously described procedure (Floreani *et al*., 1997). Freshly prepared extracts were treated for a derivatization with 2-vinylpyridine at room temperature for 60 min. Two microliter of 2-vinylpyridine was added to 96 μl of cellular extract. Then, 80 μl of 0.1 M phosphate buffer (pH 7.4)~10 mM EDTA, 10 μl of 4.8 mM NADPH and 5 μl of 4 mM DTNB were added to the reaction mixture for assaying GSSG.

Determination of intracellular ROS

For analysis of intracellular ROS, the redox-sensitive fluorescent probe DCFH-DA was used as previously described (Royall and Ischiropoulos, 1993). Cells were incubated with 5 μM DCFH-DA for 30 min at 30°C. The harvested cells were immediately analyzed by flow cytometry.

Results and Discussion

Until recently, the two peroxiredoxin members, named Tpx1 and Prx, were identified and characterized from the fission

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             agttcctagctgatggatccgttctacaattgacgaacaaaagatatttgaaatttgtta        60
             ataaattaattgttattttatttatatttctgtaacgctgtcgagacggacaatcagaga       120
             aaatgctgttacagtttacgaaagaccatttttaaagccatactagtaaaaacgacacc       180
             atgttaataatgatttgactagaaattttatgaaagaaaacattctttagtcatagtagg       240
             atgcaaagctattccgattgtataagcaggggaagatcggttccctatcgatcgtttgaa       300
             aggtcagctaaatacgctaaatacgattttacgggatcacattggtaatgttgtaaagta       360
             atatcaaagcgaagctaacaaattggtaatatttatgtcgtgattcttcactacctgtac      420
             tttcttcctacaccagaaagaaaatctcaggtgttattgtaaaattgttttttattttggt      480
             accgaaattctgaaagttgagtagtaaaaagttcagagtacttcatcctgaacagagttt      540
             cactactttgatcattttatctagcctttacaagttaaagcgatttaatactgatcagca      600
             ttgacaccaacgtttttggttttacaggtgttgtttagaatataaatttatatactgcag      660
             ctctgcatcaacctttctccttccctctagcattttcttgcacactttaagcctgtcaa       720
             tggacgcccccagaagatctagcagattggcagccaagattgcaaacgtcttggatagca     780
     1    M   D   A   P   R   R   S   S   R   L   A   A   K   I   A   N   V   L   D   S
             aaggaacgattattcctgaagctgctccagtaatgctgaaaaagcctgcaaaagatgaat     840
    21    K   G   T   I   I   P   E   A   A   P   V   M   L   K   K   P   A   K   D   E
             cagttgattcaactattcaagtgggagacgtaattcccgatatcactttgcctgacgaag    900
    41    S   V   D   S   T   I   Q   V   G   D   V   I   P   D   I   T   L   P   D   E
             atggaacttccattcgacttcgcgatattactgcaaataaagggcttgtaattttttgcct    960
    61    D   G   T   S   I   R   L   R   D   I   T   A   N   K   G   L   V   I   F   A
             acccaaaagctagtactcctggctgcacgaaacaaggttgtggttttcgtgataactatc    1020
    81    Y   P   K   A   S   T   P   G   C   T   K   Q   G   C   G   F   R   D   N   Y
             ctaagatacaggcctctgattatgaagtttttagggttgtctttcgatactagcaaagctc   1080
   101    P   K   I   Q   A   S   D   Y   E   V   L   G   L   S   F   D   T   S   K   A
             aaaaagctttcaaggataagcaaaactttccttatcatttgctttcggatcctaaaggtg    1140
   121    Q   K   A   F   K   D   K   Q   N   F   P   Y   H   L   L   S   D   P   K   G
             agctaattaaaaagttaggcgctgagaagcctggtggtggaaagctgtttagaagccatt    1200
   141    E   L   I   K   K   L   G   A   E   K   P   G   G   G   K   L   F   R   S   H
             ggatctttgagaaaggtactggcaaatgcatagtgaaggaaattgacatatcacctcttg    1260
   161    W   I   F   E   K   G   T   G   K   C   I   V   K   E   I   D   I   S   P   L
             tcagtgttgacaaagcctttgctgtaatcactgattctgaaccttagttgacaaattgtc    1320
   181    V   S   V   D   K   A   F   A   V   I   T   D   S   E   P   *
             tacgctcattgtcaaatttggaggatagtctacatgcctattgctatatgattcatgatg    1380
             aaattacactttttttatgtcttgctacaatgtgcttcaaccc                      1422
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Fig. 1. The nucleotide and deduced amino acid sequences of the *S. pombe* gene encoding Bcp. The nucleotides are numbered from 5' to 3' in the right margin, whereas the amino acid sequence is numbered from N-terminal to C-terminal end in the left margin. The stop codon is represented by an asterisk.

yeast *S. pombe* (Veal *et al.*, 2004; Kang *et al.*, 2008). In this study, *S. pombe* Bcp, a third peroxiredoxin member in *S. pombe*, was identified to play a protective role against stressful conditions.

Cloning and sequence analysis
The *S. pombe* genomic DNA encoding Bcp was isolated by PCR, which resulted in the recombinant plasmid pBCP10. As shown in Fig. 1, the determined DNA sequence contains 1,422 bp, which would encode a putative protein of 195 amino acid sequence with a molecular mass of 21,164 Da. The *bcp*⁺ gene does not contain an intron. The *S. pombe* Bcp contains 3 L-cysteine residues, and is relatively rich in L-lysine, L-aspartic acid, L-isoleucine, and L-alanine. Its estimated isoelectric point is 8.65, which is relatively basic. The *S. pombe* Bcp shares 38% homology with the *S. pombe* Prx (Fig. 2A). As shown Fig. 2B, it shows the enhanced ho-

mology with the counterparts of *Saccharomyces cerevisiae* (CAA86239) and *Candida glabrata* (XP_445343). The *S. pombe* Bcp contains a cysteine residue in the GCT sequence which is conserved among various Bcps (Jeong *et al.*, 2000). The Bcps of *S. pombe*, *S. cerevisiae*, and *C. glabrata* contain the conserved sequence YP(K/R)ASTPG$\underline{C}$T(R/K)Q(A/G)C GFRDN(F/Y) surrounding a conserved cysteine (underlined) residue (Fig. 2B). This region might be a common part of active sites of the Bcp proteins. However, importance of the region in the activity of the Bcp proteins may be verified through a further detailed approach. The *S. pombe* Bcp shares 22.8% identical amino acid residues and 34.2% conservative substitutions in the amino acid sequences with that of the *S. cerevisiae* Bcp. The *S. pombe* Bcp was estimated to localize in mitochondria (39.1%) using the PSORT II Prediction (experimental) software. This predictive subcellular localization was further supported by the finding

(A)

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Prx        -----MEVVFEPCFINLSKPVYVVNGIGGINEIHKLPILFSSFVLCFFGNVSGDYGYVDNVPLHI
BCP        MDAPRRSSRLAAKIANVLDSKGTIIPEAAPVMLKKPAKDESVDSTIQVGDVIPDITLPDEDGTSI
           .   : . : *:.. .:   ..   ::* .   *      .*:*   *    *:     *


Prx        SVISHFYPCLQSYNTDVIGITTDTVENICQWKAHLPRA-------LQFSLPVISDSNNEICREMG
BCP        RLRDITANKGLVIFAYPKASTPGCTKQGCGFRDNYPKIQASDYEVLGLSFDTSKAQKAFKDKQNF
            : .        :    . *.. .:: * :: : *:        * :*: . . .:    ::


Prx        MLHPLGGAKLALDAIVIIDSIG-----RRRDILPIRTTTCVSTLITAVQETVRFLAIENGRLL--
BCP        PYHLLSDPKGELIKKLGAEKPGGGKLFRSHWIFEKGTGKCIVKEIDISPLVSVDKAFAVITDSEP
           * *...* *   : :. *       * : *:   * .*: . *       .      *:
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(B)

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C. glabrata      --MELRRSTRLSAKHGNEVKDEG---PIKKKAKSVLKEKVNTKEPVKKPNTKEEKVSSDE
S. cerevisiae    MGEALRRSTRIAISKRMLEEEESKLAPISTPEVPKKKIKTGPKHNANQAVVQEANRSSDV
S. pombe         -MDAPRRSSRLAAKIANVLDSKG---------------TIIPEAAPVMLKKPAKDESVD
                     ***:*:: .        ..:.           .  . .   :   : .*


C. glabrata      AILEVGDDIPDITLQNQDGKDVSLKALAKENKVIIIFLYPKASTPGCTRQACGFRDNFDD
S. cerevisiae    NELEIGDPIPDLSLLNEDNDSISLKKITENNRVVVFFVYPRASTPGCTRQACGFRDNYQE
S. pombe         STIQVGDVIPDITLPDEDGTSIRLRDITAN-KGLVIFAYPKASTPGCTKQGCGFRDNYPK
                 :::** ***::* ::*. .: *: :: : : :::* **:*******:*.******: .


C. glabrata      LKEHG-LVLGLSHDTPAAQLKFKEKYSLPYDLLCDPTREFIGMLGAKKTPASGSIRSHFV
S. cerevisiae    LKKYA-AVFGLSADSVTSQKKFQSKQNLPYHLLSDPKREFIGLLGAKKTPLSGSIRSHFI
S. pombe         IQASDYEVLGLSFDTSKAQKAFKDKQNFPYHLLSDPKGELIKKLGAEKPGGGKLFRSHWI
                 ::      *:*** *:   :*   *:.*  *:.* .:**.**.**. *:*   ***:*.   .   :***::


C. glabrata      FADG--KLKFKRLKISPEISVADGKKEVLELAKQYSN-
S. cerevisiae    FVDG--KLKFKRVKISPEVSVNDAKKEVLEVAEKFKEE
S. pombe         FEKGTGKCIVKEIDISPLVSVDKAFAVITDSEP-----
                 * .*   *   .*.:.*** :** ..   : :
```

Fig. 2. Alignment of the putative amino acid sequence of the *S. pombe* Bcp (this study) with other similar sequences. The putative amino acid sequence of the Bcp is aligned with that of one known peroxiredoxin (Kang *et al.*, 2008), Prx, in the same organism (A). It is also aligned with amino acid sequences of two other Bcps from *Candida glabrata* (XP_445343) and *Saccharomyces cerevisiae* (CAA86239) (B). The asterisks, colons and periods indicate identical, conserved substitutions and semi-substitutions, respectively. Gaps, shown as short bars, were introduced for optimal alignment.

that the *bcp*[+] gene contained a putative mitochondrial targeting signal at the N-terminal region, MDAPRRSSRLA AKIANVL, which is very similar to the consensus signal sequence.
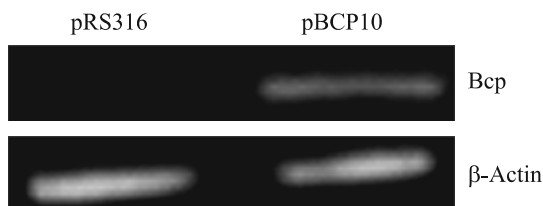
pRS316   pBCP10

Bcp

β-Actin

Fig. 3. Expression of the cloned *bcp*[+] gene in *S. pombe*. The *bcp*[+] mRNA level in *S. pombe* KP1 cells harboring pBCP10 or pRS316 was detected using RT-PCR.

Expression

To determine whether the cloned *bcp*[+] gene is functional or not, the *bcp*[+] mRNA levels in the exponential *S. pombe* KP1 cells harboring pRS316 or pBCP10 were detected using RT-PCR. As shown in Fig. 3, the *bcp*[+] mRNA level in the pBCP10-containing cells was significantly higher than that in the pRS316-containing cells, which suggested that the cloned *bcp*[+] gene was functioning. Since the *S. pombe* Bcp has not been purified so far, the specific antibody for it has not been prepared. That's why we were unable to show the enhanced expression of Bcp on a protein level in the pBCP10-containing cells. However, the significant increase in the *bcp*[+] mRNA level positively suggests expression of the cloned *bcp*[+] gene in the fission yeast. Taken together, the *S. pombe* Bcp is a third form of functional Prxs in *S. pombe* and at least three Prx isoforms exist in *S. pombe*.
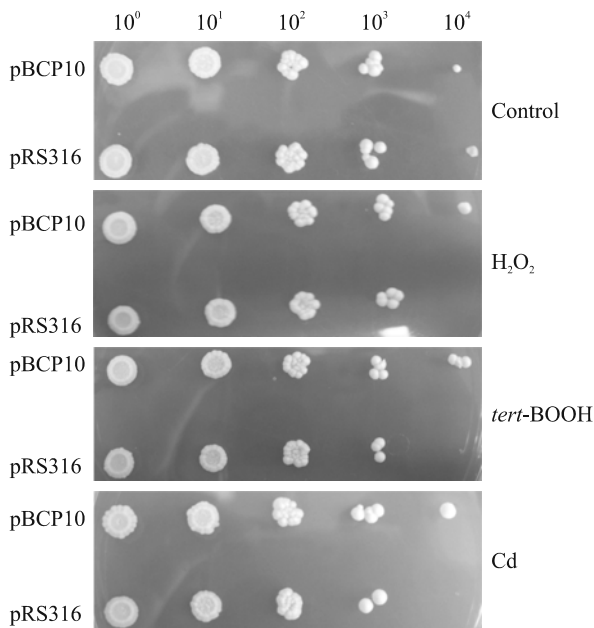
Fig. 4. Enhanced survival of the *S. pombe* KP1 cells harboring the recombinant plasmid pBCP10 on the minimal plates containing hydrogen peroxide (500 μM), *tert*-BOOH (500 μM) or cadmium chloride (25 μM). The control yeast cells contain the vector pRS316 only. The figures above the growth patterns indicate dilution factors for spotting.

Viability under oxidative stress

To assess a protective role of the *S. pombe* Bcp, the same numbers of the *S. pombe* KP1 cells harboring pBCP10 or pRS316 were spotted on the minimal medium plates with 500 μM hydrogen peroxide, 500 μM *tert*-BOOH or 25 μM cadmium chloride. As shown Fig. 4, the yeast cells harbor-
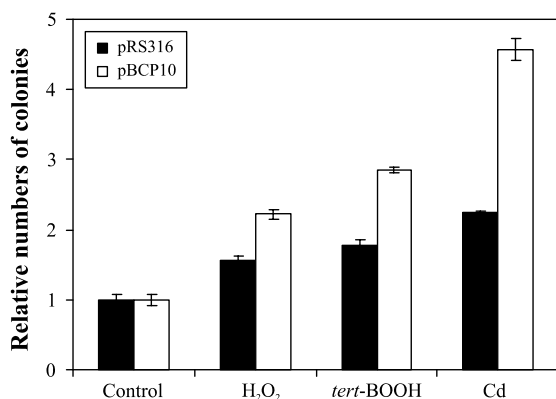
Fig. 5. Cell viabilities of the *S. pombe* cells harboring the plasmid pBCP under stressful agents, such as hydrogen peroxide (1 mM), tert-BOOH (1 mM) or cadmium chloride (50 μM). The yeast cells were obtained at 3 h after the treatments and the viable cells were counted by diluting the cultures and spreading the diluted cultures. Relative numbers of colonies were calculated by considering the viabilities of the control cultures in the absence of stressful agents as 1.

ing pBCP10 or pRS316 were able to grow at similar rates on the minimal medium plates without any stressful agents. The pBCP10-containing and control yeast cells were able to grow at similar rates in liquid media (data not shown). However, the *S. pombe* KP1 cells harboring pBCP10 gave rise to enhanced survival on the minimal media plates with hydrogen peroxide, *tert*-BOOH or cadmium chloride (Fig. 4). As shown in Fig. 5, viabilities of the *S. pombe* KP1 cells harboring pBCP10 in the liquid media were also increased compared with those of the control yeast cells, which corresponded with the survival results obtained using the solid plates. These findings might indicate that the *S. pombe* is closely involved in the viabilities of the fission yeast, especially under stressful conditions. Collectively, the *S. pombe* Bcp plays a protective role in the response to oxidative stress.

Viability under nutritional stress

Eukaryotic cells, including yeast cells, respond to nutritional stress by ultimately altering the pattern of gene expression. Cytoplasmic tRNAs accumulate in the nucleus during amino acid starvation in yeast, suggesting that tRNA nucleocytoplasmic distribution may play a role in gene expression in response to nutritional stress (Whitney *et al.*, 2007). Rpb4 and Rpb7 subunits of RNA polymerase II, which perform many cellular functions either together or independent of one another, are differentially affected during the nutritional starvation pathways of sporulation and pseudohyphae formation in *S. cerevisiae* (Singh *et al.*, 2007). In *S. pombe*, phosphatidyl(3,5)bisphosphate plays an essential role in cellular response to various stresses and in the mating pheromone signaling under starvation conditions (Morishita *et al.*, 2002). The induction of TSA1 by the Zap1 transcription factor, a central player in the response to zinc deficiency, has been shown to deal with the increased oxidative stress caused by zinc deficiency in *S. cerevisiae* (Wu *et al.*, 2007). Accordingly, participation of the *S. pombe* Bcp in cellular viability was examined under nutritional stress. The *S. pombe* KP1 cells harboring the extra copies of the bcp^+ gene showed markedly enhanced viability in minimal media with 0.4% glucose or 0.4% sucrose as a sole carbon source (Fig. 6A and B). Under nitrogen starvation, the *S. pombe* cells harboring pBCP10 showed much increased viability, compared with the stopped growth of the control yeast cells (Fig. 6C). However, the growth of the *S. pombe* KP1 cells harboring pRS315 was significantly delayed or stopped at 6 and 9 h after the treatment of 0.4% glucose (Fig. 6A) and nitrogen starvation (Fig. 6C). Overexpression of the *S. pombe* Bcp made a contribution for the better growth under those conditions. Taken together, the *S. pombe* Bcp plays a role in response to nutritional stress.

Reactive oxygen species

Reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion, and hydroxyl radical produced during metabolic reactions, cause a wide range of damage to macromolecules, resulting in genetic mutation and physiological dysfunction, and eventually cell death (Gutteridge, 1993). In the previous study, the *S. pombe* Prx was shown to play a role in maintaining low ROS level (Kang *et al.*, 2008). Then,
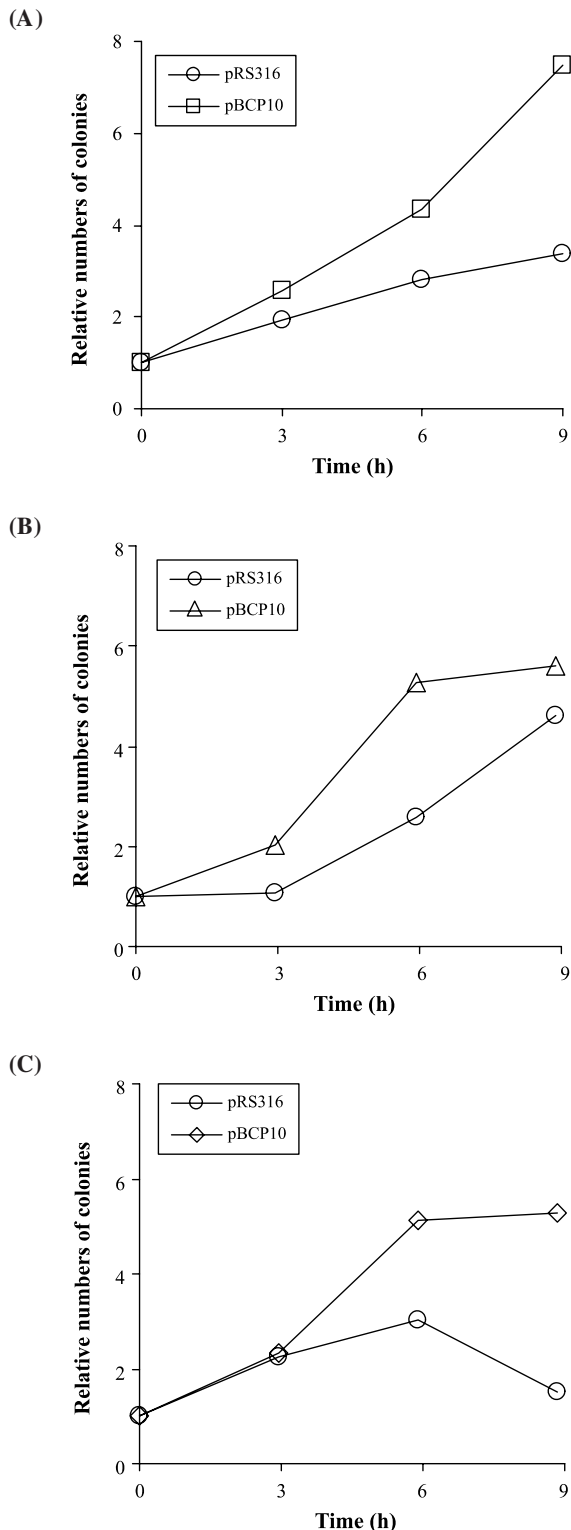
(A)

(B)

(C)

Fig. 6. Cell viabilities of the *S. pombe* cells harboring the plasmid pBCP under low concentrations of glucose (A, 0.4%) and sucrose (B, 0.4%) and under nitrogen starvation (C). The yeast cells were obtained at 3, 6, and 9 h after the treatments. The viable cells were counted by diluting the cultures and spreading the diluted cultures. Relative numbers of colonies were calculated by considering the viabilities of the corresponding cultures at time 0 as 1.
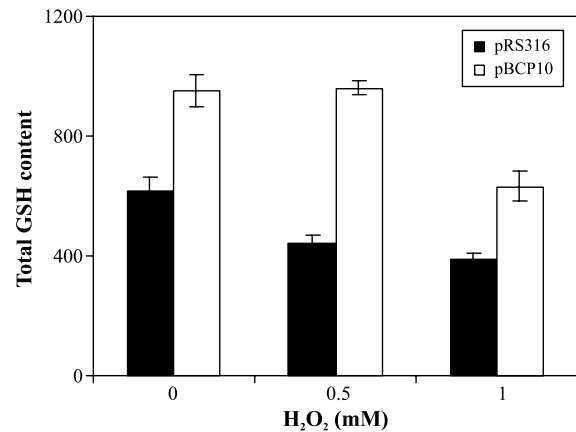
Fig. 7. Total glutathione contents in the *S. pombe* KP1 cells harboring the plasmid pBCP10 in the absence or presence of hydrogen peroxide. The control yeast cells contain the vector (pRS316) only. The yeast cells, exponentially grown in the minimal media, were treated with H_2O_2 (0.5 mM, 1 mM) for 3 h and harvested for determination of total glutathione contents. Total glutathione content was represented as μg/mg protein.

it was examined whether the Bcp is involved in maintaining low ROS level. The *S. pombe* KP1 cells harboring pBCP10 showed slightly lower ROS level than the vector control cells, which was not statistically significant (data not shown). Collectively, the *S. pombe* Bcp doesn't play a major role in maintaining low level of ROS.

Glutathione content

Glutathione, a principal thiol-containing component and a potent intracellular redox buffer, plays a crucial role in the defense of a variety of organisms against different environmental stresses. Glutathione is an inducible part of the superoxide adaptive stress response, which correlates with a decrease in the levels of intracellular oxidation (Fernandes *et al.*, 2007). The yeast cells with different reduced glutathione (GSH) contents exhibit differing stress resistances, which drives a phenotypic heterogeneity among individual cells within isogenic populations (Smith *et al.*, 2007). Dipyridyl disulfide, a highly reactive thiol oxidant, functions as electron acceptor in thiol-disulfide exchange reactions, and an increase in the ratio of oxidized to reduced glutathione (GSSG/GSH) results in an increased sensitivity to dipyridyl disulfide (López-Mirabal *et al.*, 2007). Accordingly, total glutathione (GSH+GSSG) content and the ratio of oxidized to reduced glutathione (GSSG/GSH) closely link with yeast's defense against various kinds of stresses. As shown in Fig. 7, the pBCP10-containing *S. pombe* cells contained higher total glutathione level than the vector control cells. In the treatment of hydrogen peroxide (0.5 mM, 1 mM), the higher total glutathione level in the pBCP1-containing cells was maintained (Fig. 7). The total glutathione/oxidized glutathione ratio was also higher in the pBCP10-containing cells than in the vector control cells (Fig. 8). An increase in the total glutathione/oxidized glutathione ratio may indicate an increased resistance against environmental stresses. There were not significant differences in γ-glutamylcysteine syn-
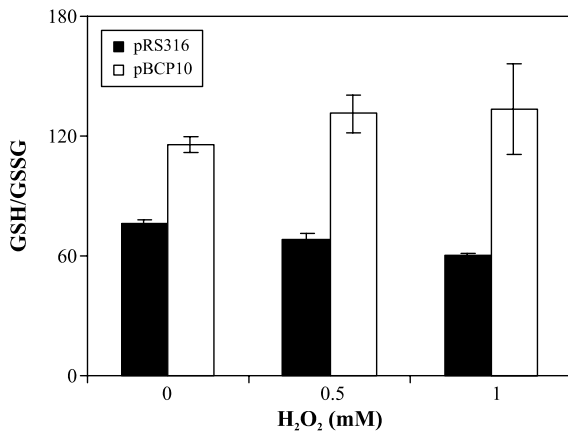
Fig. 8. Total glutathione/oxidized glutathione ratio in the *S. pombe* KP1 cells harboring the plasmid pBCP10 in the absence or presence of hydrogen peroxide. The yeast cells, exponentially grown in minimal media, were treated with H_2O_2 (0.5 mM, 1 mM) for 3 h and harvested for determining total glutathione/oxidized glutathione ratio.

thetase and glutathione synthetase mRNA levels between pBCP- and pRS316 cells (data not shown). This implies that the increased GSH content in the pBCP-containing cells might not originate from the enhanced synthesis of GSH. The *S. cerevisiae* Bcp may also be involved in the total glutathione level and the total glutathione/oxidized glutathione ratio.

In this work, an *S. pombe* gene encoding a third Prx, a homologue of Bcp, was cloned and characterized. The *S. pombe* BCP plays a protective role in the response to oxidative and metabolic stresses. Interestingly, the *S. pombe* Bcp has been shown to have a relation with the total glutathione content and the total glutathione/oxidized glutathione ratio in the fission yeast. With up-regulation of total and reduced glutathione levels, the *S. pombe* Bcp may play a role in the fission yeast under stressful conditions. In other words, since the total and reduced glutathione levels are preserved on higher levels in the overexpressed cells, the yeast cells may have enhanced protective capacities against a variety of stresses. However, how overexpressed Bcp is able to regulate total and reduced glutathione levels in the fission remains elusive so far. One possibility is that overexpressed Bcp can spare glutathione in the fission yeast under normal and stressful conditions. However, the precise mechanism how the *S. pombe* Bcp modulates the total glutathione content and the total glutathione/oxidized glutathione ratio in *S. pombe* remains elusive. The *S. pombe* Bcp might exhibit some of its cellular functions through up-regulation of glutathione biosynthesis.

Acknowledgements

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