Short communication

Dictyostelium proteins bearing motifs conserved in penicillin-binding proteins and beta-lactamases

Yosuke UMEDA, Kosuke NOMOTO, Takeshi YONEMICHI, Kentaro TAMURA and Hiro YASUKAWA*

Graduate School of Science and Engineering, University of Toyama, Toyama 930-8555, Japan.

Key words: *Dictyostelium discoideum*, LACTB, penicillin-binding protein, beta-lactamase

SUMMARY

The soil-dwelling eukaryotic microorganism *Dictyostelium discoideum* encodes a protein bearing SNTK, SNN and KTG sequences (DDB0168572) and a protein bearing SISK and HTG sequences (DDB0233562). These sequences are similar to SXXK, S(Y)XN and K(H)T(S)G sequences, which are motifs conserved in penicillin-binding proteins and beta-lactamases. RT-PCR analysis showed that *D. discoideum* expressed genes for DDB0168572 and DDB0233562 in the growth phase and had decreased expression levels of those genes in the developmental phase. A mutant in which the gene for DDB0168572 was disrupted grew and developed as did the parental strain, indicating that DDB0168572 is dispensable for *D. discoideum*. Loss of DDB0233562 is thought to be fatal to *D. discoideum* because no gene-disruption mutant was established.

Horizontal transfer and evolutional changes of genes have become recognized as a mechanism of genome evolution (Iyer et al., 2004; Jain et al., 2002; Richards et al., 2003). Transferred genes have evolved and proteins encoded play important roles in the host. Some of these proteins would remain as domains conserved in bacterial proteins. Finding and analyzing eukaryotic genes encoding such proteins are important to reveal genome evolution. Database searches have shown that eukaryotes encode proteins bearing SXXK and HTG sequences, which are similar to SXXK, S(Y)XN and K(H)T(S)G motifs conserved in bacterial penicillin-binding proteins (PBPs) and beta-lactamases (Liobikas et al., 2006; Smith et al., 2001). The

*Corresponding author
Tel:81-76-445-6875
Fax: 81-76-445-6703
E-mail: hiro@eng.u-toyama.ac.jp
Received: 14 March 2008; Accepted: 24 July 2008.

eukaryotic protein designated LACTB has been identified in various eukaryotic organisms, suggesting that it plays an important role in eukaryotes. PBPs and beta-lactamases function in synthesis of peptidoglycan and inactivation of beta-lactam antibiotics, respectively. These functions are required for bacteria but not for eukaryotes; therefore, the function of LACTB is not known.

We previously reported that the cellular slime mold *Dictyostelium discoideum* encodes a protein similar to PBP (Yasukawa et al., 2003). This protein, PscA (same as DDB0168572), bears SNTK, SNN and KTG sequences. We carried out further screening in the online informatics resource for *D. discoideum* (http://dictybase.org/) and found an additional protein bearing the motifs. The accession number of the protein is DDB0233562. Amino acid sequences around the SXXK, S(Y)XN and K(H)T(S)G motifs in LACTBs, *Escherichia coli* PBPs, *E. coli* beta-lactamases, DDB0168572 and DDB0233562 are presented in Fig. 1.

Expression levels of the genes for DDB0168572 and DDB0233562 in an axenic strain of *D. discoideum*, Ax2, were analyzed by RT-PCR. RNA samples prepared from growing cells and developing cells were used as templates. Reaction products were electrophoresed and photographed (Fig. 2). The results show that *D. discoideum* expressed the genes in the growth phase and had reduced the expression levels of the genes in the developmental phase. We obtained almost the same results from two independent experiments.

We conducted experiments to investigate the importance of DDB0168572 and DDB0233562 in *D. discoideum* by transforming Ax2 with knockout constructs of the genes that were disrupted by a gene cassette conferring blasticidin S (BS) resistance on *D. discoideum* (a *bsr* cassette; 1.35 kb in size) (Fig. 3A and B).

Ax2 was transformed by a construct to disrupt the gene for DDB0168572, cultured with BS for

```
                                SXXK motif              S(Y)XN motif          K(H)T(S)G motif

H.sapiens LACTB          157 ETVMRIASISKSLTMVAL-(141)-KPGSQFLYSTFGYTLLA-(145)-KQRHYASHTGGAVGASS 494
B.taurus LACTB           159 ETVMRIASISKSLTMVAI-(146)-KPGSQFLYSTFGYTLLA-(145)-KQRHYASHTGGAVGASS 501
M.musculus LACTB         155 ETVMRIASISKSLTMVAL-(147)-KPGSQFLYSTFGYTLLA-(145)-KQRHYASHTGGAVGASS 498
R.norvegicus LACTB       155 ETVMRIASISKSLTMVAL-(146)-KPGSQFLYSTFGYTLLA-(145)-KQRHYASHTGGAVGASS 497
G.gallus LACTB           115 ETIMRIASISKCLTMMAV-(144)-KPGSQFLYSTYGFTLLS-(145)-QQRHYASHTGGAVGASS 455
C.elegans LACTB           88 DSVMRIASISKPITATLA-( 95)-KPGSKFSYTTYGLTLAG-(135)-SNSSFFYHTGAAVGASS 369
DDB0233562               118 SSKLRVASISKALTSIGL-( 92)-KPGHYFNYSTFGYTLLG-(139)-NNTDIIYHTGNAVGGST 400
DDB0168572                87 LQAFTPASNTKLFTTISI-(239)-LNYTLLTSNNLYAETFL-( 93)-ASGIVHAKTGSMTGVNS 470
E.coli PBP1a             458 QALRQVGSNIKPFLYTAA-( 41)-LRQGLGQSKNVVMVRAM-(175)-QRRDIGGKTGTTNSSKD 725
E.coli PBP1b             503 QARRSIGSLAKPATYLTA-( 44)-LVDALTRSMNVPTVNLG-(109)-PNLHLAGKTGTTNNNVD 707
E.coli PBP1c             335 NSIRSPGSVLKPFVYGLA-( 36)-MSEALVRSLNLPAVQVL-( 98)-RVAPLAWKTGTSYGYRD 520
E.coli PBP2              323 QGVYPPASTVKPYVAVSA-( 78)-IDLAEERSGNMPTREWK-(101)-APYKIAAKSGTAQVFGL 553
E.coli PBP3              300 TDVFEPGSTVKPMVVMTA-( 34)-LTGVLQKSSNVGVSKLA-(118)-KGYRIAIKTGTAKKVGP 503
E.coli PBP4               55 QQMALPASTQKVITALAA-(226)-LKIMLKKSDNMIADTVF-( 94)-VDGKVSAKTGSLQGVYN 426
E.coli PBP5               66 DVRRDPASLTKMMTSYVI-( 48)-IRGINLQSGNDACVAMA-( 86)-SLNVDGIKTGHTDKAGY 251
E.coli PBP6               59 DEKLDPASLTKIMTSYVV-( 48)-NKGVIIQSGNDACIALA-( 86)-NLNEDGMKTGTTAGAGY 244
E.coli PBP7               63 DLVRPIASISKLMTAMVV-( 39)-LLLALMSSENRAAASLA-( 90)-NWNIQLTKTGFTNAAGH 243
E.coli DacD               56 HQQRNPASLTKLMTGYVV-( 48)-SRGLIVDSGNDACVALA-( 86)-TMNVDGLKTGHTSGAGF 241
E.coli AmpC               73 QTLFELGSVSKTFTGVLG-( 68)-APGTQRLYANSSIGLFG-(148)-VRASWVHKTGATGGFGS 340
E.coli AmpH               80 DSVVRIASLTKLMTSEML-( 78)-APGSQAAYSNLAFDLLA-(127)-GRPGIIQKTGGGGGFIT 336
```

Fig. 1. Sequence alignment of the conserved motifs in LACTBs, *E. coli* PBPs, *E. coli* beta-lactamases, DDB0168572 and DDB0233562. The conserved amino acids in the motifs are typed in white on a black background. The accession numbers of the LACTBs are P83111 (*Homo sapiens*), P83095 (*Bos taurus*), AAG37911 (*Mus musculus*), XP_217181 (*Rattus norvegicus*), NP_001025717 (*Gallus gallus*) and O62481 (*Caenorhabditis elegans*). The accession numbers of the *E. coli* proteins are P02918 (PBP1a), P02919 (PBP1b), P76577 (PBP1c), P0AD65 (PBP2), P0AD68 (PBP3), P24228 (PBP4), P0AEB2 (PBP5), P08506 (PBP6), P0AFI5 (PBP7), P33013 (DacD), P00811 (AmpC) and P0AD70 (AmpH).

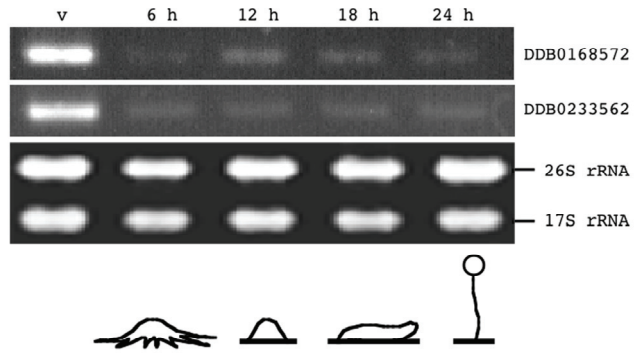

Fig. 2. Developmental expression patterns of DDB0168572 and DDB0233562. Changes in gene expression during the life cycle of *D. discoideum* were analyzed by RT-PCR. Total RNA samples extracted from growing amoebae (v) and developing cells (6 – 24 h) were used as templates. Reaction products amplified by 26 cycles (DDB0168572) and 25 cycles (DDB0233562) following reverse transcription are shown. Reaction was performed by the use of a Primescript One Step RT-PCR Kit (Takara Bio, Shiga, Japan). Primers used to amplify mRNA for DDB0168572 are 5'-GATGGTAATATTCCAAGTGCTTGGGAATGGG (P1) and 5'-CAACGCCACCATCTTCCAACAATGCTGAG (P2). The DDB0233562 mRNA was amplified by the primers 5'-CCAATGGAAACTTCAAGTAAATTAAGAGTTGCGAG TATTTC (P3) and 5'-GCTGATTCAATTACTAAACCTAATAAAGTGTAACCAAAAGTTG (P4). The EtBr staining pattern of rRNAs and illustrations of the multicellular structures are presented.

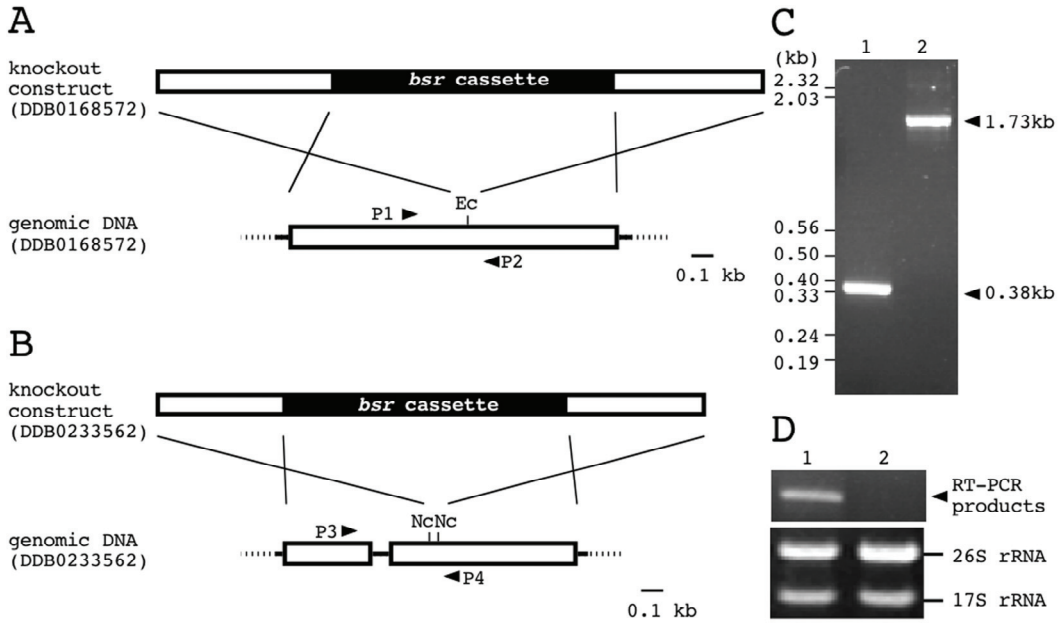

Fig. 3. Disruption of the genes for DDB0168572 and DDB0233562.
(A) Construct used in disruption of the gene encoding DDB0168572. The *bsr* cassette was inserted into the *Eco*RI site in the DDB0168572 cDNA. The gene for DDB0168572 is not interrupted by an intron. The restriction site for *Eco*RI (Ec) is indicated. Arrowheads indicate primers P1 and P2. (B) Construct used in disruption of the gene encoding DDB0233562. The *bsr* cassette was inserted into the *Nco*I site in the DDB0233562 cDNA. The genomic sequence encoding DDB0233562 is interrupted by an intron. Restriction sites for *Nco*I (Nc) are indicated. Arrowheads indicate primers P3 and P4. (C) PCR analysis. Disruption of the gene for DDB0168572 was confirmed by PCR. Genomic DNA samples purified from the cells were used as templates. The enzyme used in the reaction was ExTaq (Takara Bio, Shiga, Japan). Primers used are P1 and P2. Lanes 1 and 2 are Ax2 and the null mutant, respectively. (D) RT-PCR analysis. Disruption of the gene for DDB0168572 was confirmed by RT-PCR. Total RNA samples purified from the cells were used as templates. The EtBr staining pattern of rRNAs is presented (bottom). Lanes 1 and 2 are Ax2 and the null mutant, respectively.

selection, and subjected to analysis. Genomic DNA extracted from a selected clone was used as a template for PCR. Comparison of the sizes of the PCR products indicated that the gene in the BS-resistant strain was disrupted by insertion of the 1.35-kb fragment (Fig. 3C). Expression of the gene was examined by RT-PCR using total RNA purified from the cells. The DNA fragments were amplified from the RNA of Ax2 but not from the RNA of the BS-resistant strain (Fig. 3D). These results indicate that the gene for DDB0168572 in the clone was disrupted by the insertion of the *bsr* cassette. The gene disruption mutant we established grew and developed normally under laboratory conditions (data not shown).

Ax2 was also transformed by a construct to disrupt the gene for DDB0233562. BS-resistant cells were observed in culture medium 6 days after transformation but did not survive for more than 8 days in the medium. Three sets of the experiments were conducted, but no stable transformant was established, indicating that disruption of the gene is fatal to *D. discoideum*.

In summary, the current results indicate that DDB0168572 is dispensable for *D. disocideum*, whereas DDB0233562 is essential for this organism. The genes for DDB0168572 and DDB0233562 might have been horizontally transferred from bacteria, one of them evolving into the gene essential for the host. Further analysis of DDB0168572 and DDB0233562 is needed to reveal evolution of the genes and genome.

ACKNOWLEDGMENTS We thank members of the *Dictyostelium* cDNA project team for providing the cDNA clones for DDB0168572 (SSA619) and DDB0233562 (VFG665).

REFERENCES

Iyer, L. M., Aravind, L., Coon, S.L., Klein, D.C. and Koonin, E.V. (2004) Evolution of cell-cell signaling in animals: did late horizontal gene transfer from bacteria have a role? Trends Genet., 20, 292-299.

Jain, R., Rivera, M.C., Moore, J.E. and Lake, J.A. (2002) Horizontal gene transfer in microbial genome evolution. Theor. Popul. Biol., 61, 489 -495.

Liobikas, J., Polianskyte, Z., Speer, O., Thompson, J., Alakoskela, J.M., Peitsaro, N., Franck, M., Whitehead, M.A., Kinnunen, P.J. and Eriksson, O. (2006) Expression and purification of the mitochondrial serine protease LACTB as an N-terminal GST fusion protein in *Escherichia coli*. Protein Expr. Purif., 45, 335-342.

Richards, T.A., Hirt, R.P., Williams, B.A. and Embley, T.M. (2003) Horizontal gene transfer and the evolution of parasitic protozoa. Protist, 154, 17-32.

Smith, T.S., Southan, C., Ellington, K., Campbell, D., Tew, D.G. and Debouck, C. (2001) Identification, genomic organization, and mRNA expression of LACTB, encoding a serine beta -lactamase-like protein with an amino-terminal transmembrane domain. Genomics, 78, 12-14.

Yasukawa, H., Kuroita, T., Tamura, K. and Yamaguchi, K. (2003) Identification of the penicillin-sensitive carboxypeptidase in the cellular slime mold *Dictyostelium discoideum*. Biol. Pharm. Bull., 26, 1018-1020.