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Genome-wide screening of *Escherichia coli* genes involved in execution and promotion of cell-to-cell transfer of non-conjugative plasmids: *rodZ* (*yfgA*) is essential for plasmid acceptance in recipient cells

Naomi Kurono, Ayako Matsuda, Rika Etchuya, Rina Sobue, Yumi Sasaki, Miki Ito, Tsuyako Ando, Sumio Maeda *

Graduate School of Humanities and Sciences, Nara Women's University, Kitauoya-nishimachi, Nara 630-8506, Japan

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ABSTRACT

Acquisition of new genetic traits by horizontal gene transfer is a bacterial strategy for adaptation to the environment. We previously showed that *Escherichia coli* can transmit non-conjugative plasmids laterally in a co-culture containing strains with and without the plasmid. In this study, using the Keio collection, a comprehensive library of *E. coli* knock-out mutants for non-essential genes, we screened for genes responsible for the execution and promotion of cell-to-cell plasmid transfer in recipient cells. By stepwise screening of 'transfer-down' mutants, two essential genes and six promoting genes were obtained. One of the essential genes was *priA*, which is involved in DNA replication. This *priA* mutant was also unable to be transformed by artificial transformation methods, probably due to the deficiency of the plasmid maintenance function. The other essential gene was *rodZ* (*yfgA*), a gene involved in the regulation of rod-shaped structure of *E. coli* cells. This *rodZ* mutant was transformable by all three methods of artificial transformation tested, suggesting that this gene is essential for cell-to-cell plasmid transfer but not for artificial transformation. These are the first data that suggest that *rodZ* plays an essential role in DNA acquisition.

1. Introduction

Lateral gene transfer between bacterial cells contributes to bacterial adaptation to various environments, which eventually leads to bacterial evolution [1–3]. In human environments, however, it results in the undesirable spread of pathogenic, antibiotic resistance, or artificially engineered genes [2,4–8]. Three mechanisms of lateral gene transfer in bacteria are generally known: conjugation, transduction and transformation [2]. Conjugation and transduction involve specific apparatus, i.e. conjugative pili and phage capsids, respectively, for DNA transfer from donor to recipient cells. However, transformation is mainly performed by the recipient cells that express genetic competence for the uptake of extracellular free DNA [9,10]. Competence for transformation can be induced both naturally and artificially, but not all bacterial species develop natural competence [1,9,10].

Under natural conditions, *Escherichia coli* is not assumed to be transformable, but it is known to develop high genetic competence only under artificial conditions, such as exposure to high Ca^{2+} concentrations [11,12]. However, recent reports have shown that *E. coli* can express modest genetic competence in certain conditions that can arise in its environment [13–20]. Relevant to these findings, we found that spontaneous lateral transfer of non-conjugative plasmids occurred in an *E. coli* cell-mixed culture [21–23]. Based on the results of subsequent analyses, we hypothesized that this cell-to-cell plasmid transfer results from the transformation of plasmid DNA released from co-cultured cells [23]. However, detailed molecular mechanisms of this process, including the genes involved, are unclear.

In this study, we used the Keio collection, established by Baba, et al. [24], to explore the genes responsible for the execution and promotion of cell-to-cell plasmid transfer in a culture. The Keio collection is a comprehensive library of *E. coli* knock-out mutants for 3985 non-essential genes, which constitute 90% of all the genes in the *E. coli* K-12 genome. This collection was previously used for several genome-wide screenings of genes involved in various cell functions [25–27]. To apply the Keio collection for the screening of essential or promoting genes in cell-to-cell plasmid transfer, we first constructed a 96-well microplate assay system for cell-to-cell plasmid transfer. Then, using the Keio strains as plasmid recipients in this assay, we screened 'transfer-down' mutants (henceforth referred to as 'down' mutants) that lost the ability of plasmid acquisition. The knock-out genes in these 'down' mutants

Abbreviations: cam, chloramphenicol; tet, tetracycline; kan, kanamycin; PEG, polyethylene glycol; TSB, tryptic soy broth.

* Corresponding author.
E-mail address: smaeda@cc.nara-wu.ac.jp (S. Maeda).

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were considered to be candidates for the essential or promoting genes for cell-to-cell plasmid transfer. Here, we present the results of the screening and analyses for down mutants. These data indicate that *rodZ (yfgA)* plays an essential and specific role in the DNA acceptance in cell-to-cell plasmid transfer in *E. coli*.

2. Materials and methods

2.1. E coli strains, plasmids and materials

CAG18439 [28] (MG1655 derivative; F^-, λ^-, *lacZ118(Oc), lacI3042::Tn10(tet^r), rph-1*), BW25113 [24,29] (F^-, *rrnB, ΔlacZ4787, HsdR514, Δ(araBAD)567, Δ(rhaBAD)568, rph-1*), Keio strains [24] (BW25113 derivatives, Δ*(single gene)::kan^r*), pSY510 and pGBM1 were obtained from the National BioResource Project (NIG, Japan): *E. coli* (http://www.shigen.nig.ac.jp/ecoli/strain/top/top.jsp). Plasmids pHSG399-F6 (*cam^r*, pMB1 origin) and pHSG299-Cm (*cam^r*, pMB1 origin) were constructed as described previously [30]. Chloramphenicol (cam), tetracycline (tet), polyethylene glycol (PEG; molecular mass = 8000) and Luria–Bertani powder (LB, Lennox) were purchased from Sigma. Tryptic Soy Broth (TSB) was procured from Becton, Dickinson. Distilled water (DNase- and RNase-free, molecular biology grade) and kanamycin (kan) were obtained from Invitrogen. Microplates (96-well) and pin replicators were purchased from Nippon Genetics. Nylon66-membrane filter (pore size: 0.45 μm, Biodyne A) was procured from Pall. Agar powder (guaranteed reagent grade) and other general reagents were obtained from Wako.

2.2. Screening of down mutants for cell-to-cell plasmid transfer using 96-well microplates

We constructed a screening system for cell-to-cell plasmid transfer using 96-well microplates with some modifications [21–23] to screen down mutants from among the Keio strains as recipient cells. Screenings were performed in six steps, as described below.

For the 1st to 3rd screenings, transformants were selected twice in liquid media containing two antibiotics in 96-well microplates. Plasmid-donor cells [CAG18439 harboring pHSG399-F6 (or pHSG299-Cm only for the 3rd screening)] were pre-cultured in 10 mL of LB broth (tet: 75 μg/mL, cam: 100 μg/mL) at 37 °C for 22 h. Cultured donor cells were recovered by centrifugation and suspended in 7 mL LB broth. Recipient cells (each Keio strain) were pre-cultured at 37 °C for 22 h in 200 μL LB broth (kan: 75 μg/mL) in wells of microplates, recovered by centrifugation and suspended in 50 μL of the above donor cell suspension. Five microliters of each mixture of Keio and donor cells was inoculated on the surface of TSB agar (1.5%) prepared in wells of microplates and cultured in quadruplicates at 25 °C (duplicates) and at 37 °C (duplicates) for 16 h. The cultured cells in wells were suspended in 100 μL LB broth, and various volumes (5 μL in the 1st screening; 20 μL in the 2nd and 3rd screenings) of the suspensions were transferred to 100 μL of the 1st selection LB broth containing cam (for plasmids; 100 μg/mL) and kan (for Keio strains; 75 μg/mL) and cultured at 37 °C for 16 h. Small amounts (~0.5 μL) of the resulting 1st selection cultures were inoculated with a 96-pin replicator in 100 μL of the 2nd selection medium containing the same antibiotics and cultured at 37 °C for 16 h. The turbidities (OD_{600} values) of the resulting 2nd selection cultures were measured using a microplate reader (Multiskan JX, Thermo Fisher Scientific). Samples that showed no apparent growth in all quadruplicates were regarded as down mutants.

For the 4th–6th screenings, transformants were selected by plating onto agar media. In the 4th and 5th screenings, the steps from pre-culture to mixed culture were the same as those in the 1st and 2nd screenings, but transformants were selected and counted quantitatively on LB agar plates containing antibiotics. In the 5th and 6th screenings, lower concentrations (cam: 50, 25 or 12.5 μg/mL) of antibiotics were used to exclude 'pseudo-down' mutants that had become more sensitive to antibiotics. In the 6th screening, mixed culture was performed in a colony biofilm on TSB agar (1.5%) prepared in a polystyrene plate (ø 90 mm), a culture system more sensitive for the detection of cell-to-cell plasmid transfer, as described previously [21]. Transformants were then colonized and counted on LB agar plates containing two antibiotics (cam: 50, 25, or 12.5 μg/mL and kan: 75 μg/mL). Three Keio mutants (*allD*, *ivy* and *prpD*) that showed plasmid transfer frequency around the average value were used as control strains.

2.3. Artificial transformation of down mutants

Artificial transformation of the screened Keio mutants with purified plasmids was performed by three typical methods, i.e. $CaCl_2$ method [12,31], PEG method [31,32], and electroporation [32]). BW25113, the original strain of the Keio mutants, was used as the control.

2.4. Data analyses for screened genes

Information on the screened genes was obtained from the following databases: PEC (http://www.shigen.nig.ac.jp/ecoli/pec/index.jsp), Uniprot (http://www.uniprot.org/), Echobase (http://www.york.ac.uk/res/thomas/index.cfm), GenoBase (http://ecoli.naist.jp), and Ecocyc (http://ecocyc.org/).

3. Results and discussion

3.1. Screening of down mutants for cell-to-cell plasmid transfer using 96-well microplates

To screen down mutants for cell-to-cell plasmid transfer from 3985 strains of the Keio collection, we constructed a screening system using the 96-well microplate, as described in Section 2. Using this system, 6-step screening was performed with different screening conditions, as described in Section 2. Finally, eight down mutants were screened. Most probable pseudo-down mutants that had become more sensitive to antibiotics were excluded by the 5th and 6th screenings, in which lower concentrations of antibiotics were used. The frequencies of plasmid transfer in the eight screened mutants in the 6th screening are shown in Fig. 1. Plasmid transfer in all those mutants was significantly reduced compared with that in the controls.

These down mutants were classified into two groups, E and P (Table 1). Group E mutants yielded no transformants in any of the 6-step screening; therefore, the two genes (*priA* and *rodZ = yfgA*) knocked out in the E mutants were considered to be the essential genes for cell-to-cell plasmid transfer. Cell-to-cell plasmid transfer experiments using another donor strain (MG1655) and different plasmids [pSY510 (p15 origin) and pGBM1 (pSC101 origin)] confirmed this hypothesis (data not shown). On the other hand, group P mutants showed reduced transfer frequencies; therefore, the six genes knocked out in the P mutants (*aceF, rnt, smpB, tonB, ycjU* and *ygcO*) were estimated to be genes that promoted cell-to-cell plasmid transfer.

3.2. Artificial transformation of down mutants

To further characterize the eight down mutants, we examined whether they were transformable by general methods of artificial

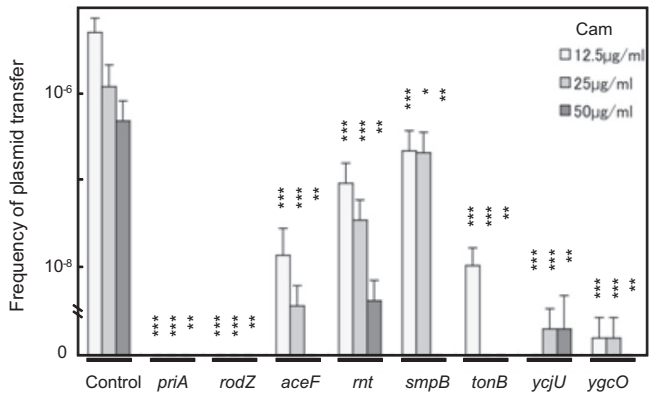

Fig. 1. Frequency of cell-to-cell plasmid transfer in screened mutants. Cell-to-cell plasmid transfer was performed with eight screened mutants and control strains as recipient cells. Data are presented as the mean and standard deviation. ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, $n = 3$, compared with control; *t*-test). Three Keio mutant strains (*allD*, *ivy* and *prpD*) that showed plasmid transfer frequency close to the average value were used as the control strains.

transformation. Using the same plasmid (pHSG399-F6) as that used in almost all the above screenings, artificial transformation was performed using the three typical protocols ($CaCl_2$ method, PEG method and electroporation) (Table 2). Most of the down mutants showed apparent normal transformability by each of the methods used, suggesting that these genes are specifically involved in plasmid acceptance in cell-to-cell plasmid transfer. The *rodZ* mutant showed lower transformability, especially with the $CaCl_2$ method, but did not completely lose its transformability. This suggests that *rodZ* is not essential for artificial transformation and plasmid maintenance. On the other hand, the *priA* mutant could not accept plasmids by any of the methods tested, suggesting that *priA* is commonly required in artificial transformation and cell-to-cell plasmid transfer. The *ycjU* mutant was transformed by the PEG method and electroporation but not by the $CaCl_2$ method, suggesting that this gene may be commonly involved in cell-to-cell plasmid transfer and artificial transformation by the $CaCl_2$ method, but not by the PEG method or electroporation. Two E group mutants were further tested with other plasmids (such as pSY510 and pGBM1), and the results were essentially the same as those shown in Table 2 (data not shown), thereby confirming the above findings.

3.3. Data summary of the screened genes

Table 1 also shows a summary of the known features of the E and P genes. The gene products localize in the cytoplasm (*priA*, *aceF*, *rnt*, *smpB*, *ycjU* and *ygcO*) and inner membrane (*rodZ* and *tonB*). The estimated functions of the gene products were as follows: transport (*tonB* and *ygcO*), metabolism and synthesis (*aceF* and *ycjU*), RNA modification and translation (*rnt* and *smpB*), DNA replication, recombination and repair (*priA*) and envelope formation (*rodZ*).

Table 2
Artificial transformation of down mutants.

Strain	Transformation frequency		
	$CaCl_2$	PEG	Electroporation
BW25113 (control)	++++	+++++	+++++
priA	–	–	–
rodZ	+	+++	+++
aceF	+++	+++++	++++
rnt	+++	+++++	++++
smpB	+++++	+++++	++++++
tonB	+++++	++++++	++++++
ycjU	–	++++	++
ygcO	++	++++	+++++

Transformation frequency (mean, $n = 3$) in each strain is shown in decimal ranges as follows: ++++++, 1E-4 to 1E-5; +++++, 1E-5 to 1E-6; ++++, 1E-6 to 1E-7; +++, 1E-7 to 1E-8; ++, 1E-8 to 1E-9; +, 1E-9 to 1E-10; –, not detected (below detection limit).

3.4. Estimated roles of the screened genes

In this study, we screened eight genes that were estimated to be essential or promoting genes for cell-to-cell plasmid transfer in *E. coli* as recipients. Although we did not obtain any clear explanations for the molecular mechanism of this phenomenon, several interesting results were obtained. The most interesting result was the essential involvement of *rodZ* in plasmid acquisition. RodZ is a Type II inner membrane protein and is estimated to be involved in the regulation of rod-shaped structure in *E. coli* cells [33–35]. Our result is the first to suggest a role for RodZ in plasmid acquisition. Interestingly, a recent study by Sanchez-Torres et al. [25] showed that RodZ is also involved in the production of extracellular DNA (or DNA secretion) in *E. coli*. RodZ protein may therefore play a dual role in the passage of DNA molecules across the cell surface structure of *E. coli*, although the detailed molecular mechanism is unknown. Moreover, RodZ protein possesses a helix-turn-helix DNA-binding motif in its cytoplasmic domain [33–35], thereby indicating its potential DNA-binding activity. Since knock-out of the *rodZ* gene did not destroy the ability for plasmid acceptance by artificial transformation (Table 2), RodZ is probably an essential component involved in a process specific to cell-to-cell plasmid transfer. On the other hand, decreased transformability was also observed by $CaCl_2$ method in the *rodZ* mutant (Table 2).

Table 1
Essential (E) and promoting (P) genes that were screened in this study and their features.

Gene name	Role in plasmid transfer	Product location	Category of product function	Product function & feature
rodZ (yfgA)	E	IM	Envelope formation	Regulation of rod cell shape, helix-turn-helix motif
priA	E	C	DNA replication, recombination & repair	DNA replication, helicase, plasmid maintenance
aceF	P	C	Metabolism, Synthesis	Dihydrolipoamide acetyltransferase, part of pyruvate dehydrogenase complex
rnt	P	C	RNA modification & translation	Ribonuclease T, tRNA processing
smpB	P	C	RNA modification & translation	SsrA RNA-binding, component of the trans–translation process
tonB	P	IM	Transport	iron ion transport, part of energy transduction complex with ExbB and ExbD
ycjU	P	C	Metabolism, Synthesis	Putative beta-phosphoglucomutase
ygcO	P	C	Transport	Ferredoxin-like, iron-sulfur cluster binding

Product location: C, cytoplasm; IM, inner membrane.

It is therefore possible that RodZ may play some part in Ca^{2+}-mediated DNA uptake.

Another essential gene, *priA*, is known to be involved in DNA replication and plasmid maintenance [36–38]. This deficient mutant was reported to be unable to maintain plasmids in cells [38]. Consistent with this result, we were unable to transform the *priA* Keio mutant with plasmids by any artificial transformation method used here. The necessity of *priA* in cell-to-cell plasmid transfer can therefore be explained by this mechanism.

The present results and existing knowledge about these genes do not clarify the roles of the other six promoting genes in cell-to-cell plasmid transfer. This may be because of the indirect involvement of these genes in plasmid acquisition. Alternatively, unidentified functions of these genes may play important roles in this process.

In *E. coli*, no apparent genes have been reported that are directly involved in natural and artificial transformation, although Finkel and Kolter [39] proposed that several competence gene homologues in *E. coli* can work to take up and utilize extracellular DNA as nutrient. However, these genes were not screened in our experiments. This is not unreasonable, because their gene products are thought to work only under nutrient-poor conditions [39] and our experiments were performed under nutrient-rich conditions. We think that another mechanism might be operating in cell-to-cell plasmid transfer. In this respect, although we recently suggested that cell-to-cell plasmid transfer occurs through a type of transformation [23], the necessity for a cell-to-cell supply of DNA indicates that cell-to-cell plasmid transfer is not a simple transformation, as suggested previously [23]. Therefore, we do not exclude the possibility that other mechanism(s) may be involved. Other recent studies have suggested that there may be several variations of the three known mechanisms in addition to novel mechanisms (e.g. transfer mediated by membrane vesicles [40] or virus-like particles [41,42] and conjugation-like transfer mediated by nanotubes [43]). However, further study is required to reveal the roles of these genes in plasmid acquisition as well as the overall mechanism of cell-to-cell plasmid transfer.

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References

[1] M.G. Lorenz, W. Wackernagel, Bacterial gene transfer by natural transformation in the environment, Microbiol. Rev. 58 (1994) 563–602.
[2] F. Bushman, Lateral DNA Transfer, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 2002.
[3] C.M. Thomas, K.M. Nielsen, Mechanisms of, and barriers to, horizontal gene transfer between bacteria, Nat. Rev. Microbiol. 9 (2005) 711–721.
[4] P. Keese, Risks from GMOs due to horizontal gene transfer, Environ. Biosafety Res. 7 (2008) 123–149.
[5] B.G. Kelly, A. Vespermann, D.J. Bolton, Gene transfer events and their occurrence in selected environments, Food Chem. Toxicol. (2008), http://dx.doi.org/10.1016/j.fct.2008.06.012.
[6] B.G. Kelly, A. Vespermann, D.J. Bolton, Horizontal gene transfer of virulence determinants in selected bacterial foodborne pathogens, Food Chem. Toxicol. (2008), http://dx.doi.org/10.1016/j.fct.2008.02.007.
[7] A. Pontiroli, A. Rizzi, P. Simonet, D. Daffonchio, T.M. Vogel, et al., Visual evidence of horizontal gene transfer between plants and bacteria in the phytosphere of transplastomic tobacco, Appl. Environ. Microbiol. 75 (2009) 3314–3322.
[8] P.S. Duggan, P.A. Chambers, J. Heritage, J.M. Forbes, Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent, FEMS Microbiol. Lett. 191 (2000) 71–77.
[9] D. Dubnau, DNA uptake in bacteria, Annu. Rev. Microbiol. 53 (1999) 217–244.
[10] I. Chen, D. Dubnau, DNA uptake during bacterial transformation, Nat. Rev. Microbiol. 3 (2004) 241–249.
[11] M. Mandel, A. Higa, Calcium-dependent bacteriophage DNA infection, J. Mol. Biol. 53 (1970) 159–162.
[12] D. Hanahan, Studies on transformation of *Escherichia coli* with plasmids, J. Mol. Biol. 166 (1983) 557–580.
[13] B. Baur, K. Hanselmann, W. Schlimme, B. Jenni, Genetic transformation in freshwater: *Escherichia coli* is able to develop natural competence, Appl. Environ. Microbiol. 62 (1996) 3673–3678.
[14] F. Bauer, C. Hertel, W.P. Hammes, Transformation of *Escherichia coli* in foodstuffs, Syst. Appl. Microbiol. 22 (1999) 161–168.
[15] S.D. Tsen, S.S. Fang, M.J. Chen, J.Y. Chien, C.C. Lee, et al., Natural plasmid transformation in *Escherichia coli*, J. Biomed. Sci. 9 (2002) 246–252.
[16] M. Woegerbauer, B. Jenni, F. Thalhammer, W. Graninger, M. Burgmann, Natural genetic transformation of clinical isolates of *Escherichia coli* in urine and water, Appl. Environ. Microbiol. 68 (2002) 440–443.
[17] S. Maeda, N. Kakihara, Y. Koishi, Competency development of *Escherichia coli* in foodstuffs, Microbes Environ. 18 (2003) 100–103.
[18] S. Maeda, A. Sawamura, A. Matsuda, Transformation of colonial *Escherichia coli* on solid media, FEMS Microbiol. Lett. 236 (2004) 61–64.
[19] D. Sun, Y. Zhang, Y. Mei, H. Jiang, Z. Xie, et al., *Escherichia coli* is naturally transformable in a novel transformation system, FEMS Microbiol. Lett. 265 (2006) 249–255.
[20] Y. Ishimoto, S. Kato, S. Maeda, Freeze–thaw-induced lateral transfer of nonconjugative plasmids by in situ transformation in *Escherichia coli* in natural waters and food extracts, World J. Microbiol. Biotechnol. 24 (2008) 2731–2735.
[21] S. Maeda, M. Ito, T. Ando, Y. Ishimoto, Y. Fujisawa, et al., Horizontal transfer of nonconjugative plasmids in a colony biofilm of *Escherichia coli*, FEMS Microbiol. Lett. 255 (2006) 115–120.
[22] T. Ando, S. Itakura, K. Uchii, R. Sobue, S. Maeda, Horizontal transfer of nonconjugative plasmid in colony biofilm of *Escherichia coli* on food-based media, World J. Microbiol. Biotechnol. 25 (2009) 1865–1869.
[23] R. Etchuuya, M. Ito, S. Kitano, F. Shigi, R. Sobue, S. Maeda, Cell-to-cell transformation in *Escherichia coli*: a novel type of natural transformation involving cell-derived DNA and a putative promoting pheromone, PLoS ONE 6 (2011) e16355.
[24] T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K.A. Datsenko, M. Tomita, B.L. Wanner, H. Mori, Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection, Mol. Syst. Biol. 2 (2006) 2006.0008.
[25] V. Sanchez-Torres, T. Maeda, T.K. Wood, Global regulator H-NS and lipoprotein NlpI influence production of extracellular DNA in *Escherichia coli*, Biochem. Biophys. Res. Commun. 401 (2010) 197–202.
[26] O. Sharma, K.A. Datsenko, S.C. Ess, M.V. Zhalnina, B.L. Wanner, W.A. Cramer, Genome-wide screens: novel mechanisms in colicin import and cytotoxicity, Mol. Microbiol. 73 (2009) 571–585.
[27] T. Inoue, R. Shingaki, S. Hirose, K. Waki, H. Mori, K. Fukui, Genome-wide screening of genes required for swarming motility in *Escherichia coli* K-12, J. Bacteriol. 189 (2007) 950–957.
[28] M. Singer, T.A. Baker, G. Schnitzler, S.M. Deischel, M. Goel, et al., A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*, Microbiol. Rev. 53 (1989) 6408–6411.
[29] B.J. Bachmann, Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12, in: F.C. Neidhardt, R. Curtiss, J.L. Ingraham, E.C.C. Lin, K.B. Low, et al. (Eds.), *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, 2nd ed., ASM Press, WA, 1996, pp. 2460–2488.
[30] R. Sobue, N. Kurono, R. Etchuya, S. Maeda, Identification of a novel DNA element that promotes cell-to-cell transformation in *Escherichia coli*, FEBS Lett. 585 (2011) 2223–2228.
[31] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: a Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989.
[32] C.T. Chung, S.L. Niemera, R.H. Miller, One-step preparation of competent *Escherichia coli*: Transformation and storage of bacterial cells in the same solution, Proc. Natl. Acad. Sci. USA 86 (1989) 2172–2175.
[33] D. Shiomi, M. Sakai, H. Niki, Determination of bacterial rod shape by a novel cytoskeletal membrane protein, EMBO J. 27 (2008) 3081–3091.
[34] F.O. Bendezu, C.A. Hale, T.G. Bernhardt, P.A. de Boer, RodZ (YfgA) is required for proper assembly of the MreB actin cytoskeleton and cell shape in *E. coli*, EMBO J. 28 (2009) 193–204.
[35] S.A. Alyahya, R. Alexander, T. Costa, A.O. Henriques, T. Emonet, C. Jacobs-Wagner, RodZ, a component of the bacterial core morphogenic apparatus, Proc. Natl. Acad. Sci. USA 106 (2009) 1239–1244.
[36] P. Nurse, R.J. DiGate, K.H. Zavitz, K.J. Marians, Molecular cloning and DNA sequence analysis of *Escherichia coli priA*, the gene encoding the primosomal protein replication factor Y, Proc. Natl. Acad. Sci. USA 87 (1990) 4615–4619.
[37] K.H. Zavitz, K.J. Marians, Dissecting the functional role of PriA protein-catalysed primosome assembly in Escherichia coli DNA replication, Mol. Microbiol. 5 (1991) 2869–2873.

[38] E.H. Lee, A. Kornberg, Replication deficiencies in *priA* mutants of *Escherichia coli* lacking the primosomal replication n' protein, Proc. Natl. Acad. Sci. USA 88 (1991) 3029–3032.
[39] S.E. Finkel, R. Kolter, DNA as a nutrient: novel role for bacterial competence gene homologs, J. Bacteriol. 183 (2001) 6288–6293.
[40] S. Yaron, G.L. Kolling, L. Simon, K.R. Matthews, Vesicle-mediated transfer of virulence genes from *Escherichia coli* O157:H7 to other enteric bacteria, Appl. Environ. Microbiol. 66 (2000) 4414–4420.
[41] T.A. Richards, J.M. Archibald, Cell evolution: gene transfer agents and the origin of mitochondria, Curr. Biol. 21 (2011) R112–R114.
[42] L.D. McDaniel, E. Young, J. Delaney, F. Ruhnau, K.B. Ritchie, J.H. Paul, High frequency of horizontal gene transfer in the oceans, Science 330 (2010) 50.
[43] G.P. Dubey, S. Ben-Yehuda, Intercellular nanotubes mediate bacterial communication, Cell 144 (4) (2011) 590–600.