**Supplementary information**

**Materials and Methods**

(1) Strains, phage, plasmid, media, and routine methods

All bacterial strains used were derivatives of *E. coli* K12 except *E. coli* C. Strains W3110 (Bachman, 1996; Hayashi et al. 2006) and BW25113 (Datsenko and Wanner 2000) are in our and Wanner’s laboratory stock, respectively. The latter strain was used for DNA linear transformation (Datsenko and Wanner, 2000). MG1655 (Bachman, 1996; Hayashi et al. 2006) was provided by the NBRP *E. coli* stock center, NIG, Japan. Strain KHG1126 has a *dif* mutation, constructed by replacing the *dif* sequence with the *cam* gene of W3110 strain (unpublished). For *tus*' mutation, *tus::Tn3* (#13) in the KHG 1438 strain in our laboratory stock was used (Kobayashi et al., 1989). The *danA*<sup>ts</sup> (ME9273) and DNA gyrase B(ts) (ME9028:W3110 *tna::Tn10 parA110*) (Kato et al. 1989) mutants were provided by NBRP (NIG) and *parC* and *E* (topoIV) temperature sensitive mutants (C600 *parC1215* and W3110 *parE10*) (Kato et al. 1990) by J. Kato. Phage N15 (Ravin and Shulga 1970), its host *E. coli* C and the N15 lysogen were provided by Dr. N. Ravin. P1*vir* was from our laboratory stock. Plasmid pBAD24 (Guzman et al. 1995) was provided by S. Yasuda (The cloning vector collection, NIG). Media and *E. coli* genetic methods were as described (Miller 1992). DNA
manipulations, such as extraction, DNA digestion, cloning and sequencing were performed as previously described (Sambrook et al. 1989).

(2) Insertion of the tos-Km fragment into *E. coli* chromosome

Essentially the linear DNA transformation method (Datsenko and Wanner 2000) was used. The tos-Km region inserted into pHSG398 (Takara Shuzo Co) was amplified by PCR using a pair of primers which had pUC universal 20mer primers with 50mer tails flanking the chromosomal region to be inserted. The PCR product was transformed to BW25113, Kmr transformants were selected, the tos-Km fragment was confirmed to be integrated into the correct site by PCR and then the tos-Km region was transduced into MG 1655 and W3110 by P1 phage.

(3) Construction and assembly of *lacO* and *tetO* arrays

We followed the method developed by Dr. Sherratt’s group. According to the procedures described by Lau et al. (2003), strains carrying 240 copies of the *lacO* and *tetO* array were constructed. For insertion of the *lacO* and *tetO* arrays into the two *ter* sites, termed *ter1* and *ter2* of the chromosome (see below), the plasmids pLAU43-Cm’ and pLAU44, respectively, were used. pLAU43 and pLAU44 were kindly provided by Dr. Sherratt. The Km’ gene of pLAU43 was exchanged for Cm’, which was derived from pSHG399 (Takara Shuzo Co). The *tetO* array 15 kb counterclockwise from *oriC*
was derived from \[tet\text{O}240\text{-Gm}]3908 (Lau et al., 2003). As a fluorescent-repressor supplier, one of us (H. N.) constructed a genetic unit on the chromosome, consisting of three genes; two genes tandemly located express the two fluorescent–repressor (LacI and TetR) fusing proteins from the \(ara\) promoter (\(pBAD::lacI\text{-cfp tetR-yfp}\)) and the third one is the Cm\(^{r}\) gene which can be used as a selective marker when the unit is transduced by P1 phage to another recipient. This can then be eliminated by using a helper plasmid expressing FLP recombinase. Details of this genetic unit will be published elsewhere. By transducing the operator arrays and the genetic unit using P1 phage, the following three strains were constructed: MKG296: MG1655 \([lac\text{O}240\text{-Cm}]1567+ [tet\text{O}240\text{-Gm}]3908, [tos-Km], yajR::(pBAD::lacI-cfp tetR-yfp)\) (\(tet\text{O}\) array \([oriC\) position]15 kb counterclockwise from \(oriC\), \(lac\text{O}\) array \([ter1\) position] 20 kb counterclockwise from the \(dif\) site, \(tos-Km\) fragment 3 kb counterclockwise from the \(dif\) site); MKG297: the same as MK296, not + [\(tet\text{O}240\text{-Gm}]3908 but +[\(tet\text{O}240\text{-Gm}]1607 (tet\text{O}\) array \([ter2\) position] 20 kb clockwise from \(dif\)); MKG305: the same as MK297 except for N15 lysogen. For integration of the \(lac\text{O}\) array into the \(ter1\) site of the chromosome, firstly, the two chromosomal regions (about 500 bp) flanking it were amplified by the following two primer sets: \(\text{EcoRI-NheI}\) primer, 5’-ccggaattcagtcgcggtgttcacatagac-3’ and 5’-tacctagctagcttgcactggaactttactgtg-3’
and XbaI-NdeI primer, 5’-ctagtctagacgacgaaagggagcgatg-3’ and 5’-ggaattccatatgcgcgtacgtctagagcagcccgtgagtt-3’, and inserted into the EcoRI-NheI and XbaI-NdeI sites of pLAU43-Cm’, respectively. Secondly, the contiguous region containing the two ter1 flanking chromosomal regions, the lacO arrays and the Cm’ marker, was amplified by PCR and transformed into the BW strain by Wanner’s method (Datsenko and Wanner, 2000). For the tetO array into the ter2 site, firstly, the two chromosomal regions (about 500 bp) flanking it were amplified by the following two primer sets: NheI-SpeI primer, 5’-atccggctagcttggaatgtcatgcg-3’ and 5’-ggctagtctagaggtgaaataagaaacccggtcg-3’ and XbaI-NdeI primer, 5’-ggctagtctagaggtgaaataagaaacccggtcg-3’ and 5’-caggcccccatatgaattttgccagtgcagtcac-3’ and inserted into the NheI-SpeI and XbaI-NdeI sites of pLAU44, respectively. Secondly, the contiguous region containing the two ter2 flanking chromosomal regions, tetO array and Gm’ marker, was amplified and transformed as described above.

(4) Microscopy

Cells for microscopy were grown in M9-succinate minimal medium at 37°C to OD600 of 0.2 – 0.4, when they were induced with 0.2 % L-arabinose for more 120 min. At the same time, IPTG (5 μM) and anhydrotetracycline (40ng /ml) were added in some experiments (Lau et al. 2003; Wang et al. 2005). Cells from the cultures were fixed on
poly-L-lysine coated slide glasses and then examined using DeltaVision System instrument (Applied Precision). The ECFP and EYFP foci were visualized using the 86002v2 filter set (Chroma). The images were captured and analyzed by SoftWoRx (included in DeltaVision System) and processed by Adobe Photoshop CS2.

(5) Agarose and pulse-field gel electrophoresis

Chromosomal DNA was isolated from cells in log phase or from overnight cultures, digested with \( \text{ShpI} \) for 3 hours at 37\(^\circ\)C, and electrophoresed in 1.0% and 1.5% agarose gels under neutral and alkaline conditions, respectively. The latter procedure was performed as previously described (Deneke et al. 2000). Southern hybridization was performed as described (Watanabe and Horiuchi 2005). Primer sequences for the probes, whose locations are shown in Fig. 1, were as follows:

probe 1: 5’-cccgccatcgacattacaactgaaggcaaa-3’ and 5’-agtgccagacaatttccaacgaccggta-3’;
probe 2: 5’–gcgtgatctgatccttcaactcagcaaaag-3’ and 5’-ggaaagccacgttgtgtctcaaatctctg-3’.

Both probes were about 1 kb in length.

Pulse-field gel electrophoresis was performed as previously described (Kodama et al. 2002). After digestion with \( \text{AvrII} \), the samples were subjected to PFGE in 0.5x TBE at 14\(^\circ\)C for 36 h at 6 V cm\(^{-1}\), using a 120 included angle with a 10 – 60 second linear
switch time ramp on CHEF-DRII (BIO-RAD) apparatus. After SYBR' Green I staining, the gel was used for hybridization.

(6) PCR assay for genome linearity.

The primer sequences used for the PCR assay, whose locations are shown in Fig. 1, were as follows: primer 1: 5’-gcggattttcttacaggtgtaggc-3’ and primer 2: 5’-aagtcaactcag-aagtccgtcaatccggga-3’. The PCR assay was carried out by PCR reaction [for 3 min at 94°C and 30 cycles (94°C for 30 sec, 59°C for 30 sec and 72°C for 2.5 min)] using the 50 μl reaction mixture containing 1 μl of the non-diluted genomic DNA or diluted as the template.

(7) Affymetrix Genechip analysis

RNA purification from three MG1655 derivatives, (tos), (N15), and (tos)(N15), was performed using RNeasy purification kits (Qiagen) following the manufacturer’s instructions. To monitor transcriptional profiles for each strain, Affymetrix E. coli Antisense GeneChips were used. In each case, total RNA (100-300 μg) was isolated from cells at OD 600 = 0.4-0.5; 10 μg was used for synthesis of cDNA and 8 μg of cDNA was used per chip. Labeling and hybridization procedures were performed according to the manufacturer’s instructions (http://www.affymetrix.com/support/technical/manuals.affx). Raw data files were
analyzed by the GCOS program using a single array analysis procedure (a target signal is 500 using all probe set). After analysis, we classified genes which have significant intensity value (> 50), the expression ratios for which in both \((tos)(N15)/(tos)\) and \((tos)(N15)/(N15)\) were more or less than four-fold, as having significantly different levels of expression.

References


high-level expression by vectors containing the arabinose $P_{BAD}$ promoter. *J Bacteriol* **177**: 4121-4130


Supplemental Figure 1 legend

PCR across the tos site is not successful when using the linear genomic DNA as a template. (a) EtBr-stained gels showing PCR reactions carried out using the non-diluted genomic DNA of MG1655, MG (N15), MG(tos) and MG(tos)(N15), as a template. Primer sites are shown in Fig. 1(b). (b) The same PCR reactions were carried out as in (a) using the non-diluted genomic DNA of MG(tos)(N15) and diluted genomic DNA of MG(tos), as templates. All samples contained 1 μl of the non-diluted genomic DNA of MG(tos)(N15). For details, see Materials and Methods.

Supplemental Figure 2 legend

Relative positioning of the two termini of the E. coli linear genome in the presence of the inducers. For details of the strains and experiments, see text and Materials and Methods. Experimental condition were the same as used for Strain MK305 (linear genome) in Figure 3C except for the addition of two kinds of inducers, IPTG (5 μM) and anhydrotetracycline (40ng /ml) at the same time that arabinose was added. Relative positioning of ter1 (red) and ter2 (green) can be seen and their behavior can be followed
substantially through the cell cycle.

Supplemental Figure 3 legend

Models showing locations of the two terminus sites (ter1 and ter2) on the circular and the linear genomes in new born cells. The ter1 and ter2 sites are located at – 20 kb and + 20 kb remote from the dif site. These models were made on the basis of the models proposed in Wang et al. (2005) and Niki and Hiraga (1998).
Supplementary Figure 1, Cui et al.
Supplementary Figure 3, Cui et al.