Systematic identification of synthetic lethal mutations with reduced-genome *Escherichia coli*: synthetic genetic interactions among *yoaA*, *xthA* and *holC* related to survival from MMS exposure

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Reduced-genome *Escherichia coli* strains lacking up to 38.9% of the parental chromosome have been constructed by combining large-scale chromosome deletion mutations. Functionally redundant genes involved in essential processes can be systematically identified using these reduced-genome strains. One large-scale chromosome deletion mutation could be introduced into the wild-type strain but not into the largest reduced-genome strain, suggesting a synthetic lethal interaction between genes removed by the deletion and those already absent in the reduced-genome strain. Thus, introduction of the deletion mutation into a series of reduced-genome mutants could allow the identification of other chromosome deletion mutations responsible for the synthetic lethal phenotype. We identified a synthetic lethality caused by disruption of *nfo* and *xthA*, two genes encoding apurinic/apyrimidinic (AP) endonucleases involved in the DNA base excision repair pathway, and two other large-scale chromosome deletions. We constructed temperature-sensitive mutants harboring quadruple-deletion mutations in the affected genes/chromosome regions. Using these mutants, we identified two multi-copy suppressors: *holC*, encoding the chi subunit of DNA polymerase III, and *yoaA*, encoding a putative DNA helicase. Addition of the *yoaA* disruption increased the methyl methanesulfonate (MMS) sensitivity of *xthA* single-deletion or *xthA nfo* double-deletion mutants. This increased MMS sensitivity was not suppressed by the presence of multi-copy *holC*. These results indicate that *yoaA* is involved in MMS sensitivity and suggest that YoaA functions together with HolC.

**Key words:** *Escherichia coli*, MMS sensitivity, DNA helicase, DNA polymerase III, AP endonuclease

A minimal gene set contains only those genes that are essential and sufficient to sustain a functioning cell. The minimal gene set includes not only a set of all essential genes but also functionally redundant nonessential genes involved in essential processes. It is difficult to identify such cryptic essential genes because the phenotypes resulting from their individual disruption are not detectable in the wild-type strain. However, reduced-genome strains provide useful tools for systematically identifying cryptic essential genes by taking advantage of their synthetic lethal interactions. To identify such genes, and ultimately a minimal gene set, we previously constructed a series of reduced-genome *Escherichia coli* strains by combining large-scale chromosomal deletion mutations (Hashimoto et al., 2005; Kato and Hashimoto, 2007; Iwadate et al., 2011). In the present work, using these reduced-genome strains, we identified a synthetic lethality involving the deletion of genes encoding AP endonucleases. In addition, we identified a novel DNA repair gene, *yoaA*, as a multi-copy suppressor of this synthetic lethality. YoaA may function together with the chi subunit of DNA polymerase III.

First, we constructed a large-scale deletion mutation, LD3-20-1, using a red+ derivative of the wild-type strain MG1655 (Fig. 1A). Next, we tried to introduce this deletion by P1 phage-mediated transduction into a reduced-genome strain, Δ33b, a fast-growing derivative of the strain with the largest reduction in genome size, Δ33a
However, no transductants appeared, suggesting that a gene within the chromosomal region affected by the large-scale deletion LD3-20-1 is synthetically lethal with other large-scale deletion mutation(s) present in ∆33b (Supplementary Tables S1 and S4).
To identify the responsible gene, we constructed a series of partial deletions of LD3-20-1 and tried to introduce them into Δ33b (Fig. 1A). The results revealed that nfo is responsible for the observed synthetic lethality. The nfo gene encodes DNA endonuclease IV, which is involved in DNA repair. This enzyme cleaves phosphodiester bonds at apurinic or apyrimidinic sites (AP sites) to produce new 5'-ends that are base-free deoxyribose 5-phosphate residues, and preferentially attacks modified AP sites created by bleomycin and neocarzinostatin (Levin et al., 1991; Hosfield et al., 1999).

To identify other responsible genes, we introduced the nfo disruption into other reduced-genome strains. nfo disruptants were obtained with Δ20a but not with Δ21a (Supplementary Tables S1 and S4). The Δ21a strain was constructed by introducing a large-scale chromosome deletion, LD3-17-1 (deletion location is 1,821,337–1,860,378 bp and the length is 39,042 bp; for further information, see the PEC database, http://www.shigen.nig.ac.jp/ecoli/pec/), into Δ20a, suggesting that a second gene responsible for synthetic lethality is present in the chromosomal region affected by this deletion (Supplementary Tables S1 and S4). We suspected xthA as the responsible gene in this region: it encodes DNA endonuclease VU/DNA exonuclease III, and is also involved in DNA repair (Mol et al., 1995; Shida et al., 1996). XthA is a major AP endonuclease that removes damaged DNA at cytosines and guanines by cleaving on the 3'-side of an AP site via a beta-elimination reaction. It also exhibits 3'-5'-exonuclease, 3'-phosphomonoesterase, 3'-repair diesterase and ribonuclease H activities. We tried to introduce the xthA deletion in addition to the nfo deletion into Δ20a by transduction, but did not obtain any transductants, suggesting that xthA was responsible for the synthetic lethality.

To search for further responsible genes, we introduced the nfo and xthA disruptions into other reduced-genome strains. nfo xthA double disruptants were obtained with Δ16A/K, but not with Δ17A/K (Supplementary Tables S1 and S4). The Δ17a strain was constructed by introducing a large-scale chromosome deletion, LD3-5-1, into Δ16a, suggesting that a responsible gene was present in this chromosomal region. When we introduced the nfo and xthA disruptions into other reduced-genome strains, we observed that many colonies appeared with Δ13a, but few with Δ14a. Because the Δ14a strain was constructed by introducing a large-scale chromosome deletion, LD20 (deletion location is 1,349,195–1,650,779 bp and the length is 301,585 bp; for further information, see the deletion ‘20’ in the PEC database), into Δ13a, this deletion also appeared to be responsible for the synthetic lethality.

To confirm that these four deletions were sufficient for the synthetic lethality, we tried to introduce all four deletions (Δnfo, ΔxthA, LD3-5-1 and LD20) into MG1655. When we attempted to introduce LD20 into MG1655 harboring Δnfo, ΔxthA and LD3-5-1 by transduction, we obtained no transductants containing LD20, strongly suggesting that the synthetic lethality was caused by these four deletions. To identify the responsible gene within the region of LD3-5-1, we constructed a series of partial deletions of LD3-5-1 and tried to introduce them into MG1655 harboring two (Δnfo and ΔxthA) or all three (Δnfo, ΔxthA and LD20) of the other deletions (Fig. 1B). The results suggested that deletions of uvrC and/or uvrY were responsible for the synthetic lethality of the four deletions (Δnfo, ΔxthA, LD3-5-1 and LD20), but neither of these deletions alone were sufficient for a growth defect in the presence of the Δnfo and ΔxthA deletions. The responsible gene may be uvrC, because the uvrC gene is involved in DNA repair and uvrY codes for a member of the two-component regulatory system involved in the regulation of carbon metabolism.

The synthetic lethality of the four deletions (Δnfo, ΔxthA, LD3-5-1 and LD20) was also confirmed by constructing two plasmids, miniFts-nfo+ and miniFts-xthA+, that could not replicate at high temperature. In the presence of either plasmid, quadruple-deletion mutants were obtained at 30 °C but not at 42 °C, indicating that the synthetic lethality was caused by the four deletions.

To identify other responsible genes in the deleted regions of LD3-5-1 and LD20, as well as multi-copy suppressors, we obtained temperature-resistant (tr) colonies by introducing an E. coli chromosome library constructed in the multi-copy plasmid pACYC184 (Chang and Cohen, 1978). Plasmids were extracted from 150 tr colonies, and each of them was re-introduced into the ts quadruple-deletion mutants to confirm multi-copy suppression. Among the 150 plasmids, 89 were confirmed to suppress the synthetic lethality. After elimination of three large plasmids, sequencing and PCR analyses of the resultant 86 plasmids revealed that 34 plasmids carried nfo, 34 contained xthA, 17 carried a chromosomal region including holC, and one contained the region containing yoaA. To identify the responsible multi-copy suppressor gene within the chromosomal region including holC, we constructed deletion-derivative plasmids and examined their suppression activity (Supplementary Fig. S1A). The results revealed that holC was indeed responsible for suppression. Likewise, to identify the responsible multi-copy suppressor gene within the chromosomal region containing yoaA, we constructed deletion-derivative plasmids and examined their suppression activity (Supplementary Fig. S1B). The results confirmed that yoaA was responsible.

holC and yoaA were identified as multi-copy suppressors of the synthetic lethality of the four deletions (Δnfo, ΔxthA, LD3-5-1 and LD20), and their multi-copy plasmids also suppressed the MMS sensitivity of the ΔxthA mutant (Fig. 2A). MMS is an alkylating agent and produces DNA damage (Lundin et al., 2005). To confirm the involvement of yoaA in MMS sensitivity, we introduced a
complemented by the yoaA plasmid, indicating that YoaA is involved in determining MMS sensitivity.

To investigate the possibility that yoaA, like nfo and xthA, is involved in DNA repair, we examined the MMS sensitivity of the ΔyoaA derivatives of the Δnfo and ΔxthA single-deletion mutants and the Δnfo ΔxthA double-deletion mutant. As shown in Fig. 3, the ΔxthA and Δnfo ΔxthA mutants became more sensitive to MMS upon addition of the ΔyoaA mutation. However, acquisition of the ΔyoaA mutation did not affect the sensitivity of the parental strain or the Δnfo single mutant (Fig. 3).

YoaA is a putative DNA helicase (UniProt, http://www.uniprot.org/uniprot/P76257), and HolC is the chi subunit of DNA polymerase III (Carter et al., 1993; Xiao et al., 1993a, 1993b). An interaction between YoaA and HolC was previously revealed by interactome analysis (DIP (DIP-12786N), http://dip.doe-mbi.ucla.edu/dip/Browse.cgi?ID=DIP-12786N; IntAct (P76257), http://www.uniprot.org/uniprot/P76257#interaction). To investigate functional interaction between these two proteins, we examined multi-copy suppression by holC in the yoaA disruptant. In addition, we introduced ΔxthA into the quadruple-deletion mutant (Δnfo, ΔyoaA, LD3-5-1 and LD20) and examined the effects of the holC multi-copy plasmid on the growth of the transductants (Fig. 4). Multi-copy suppression by pACYC184-holC* was observed when the yoaA gene was present, but not in cells harboring ΔyoaA. In addition, we examined the multi-copy suppression against MMS sensitivity of the ΔxthA mutant. As shown in Fig. 2A, MMS sensitivity was suppressed by the holC multi-copy plasmid when the yoaA gene was
E. coli yoaA and holC function in MMS sensitivity present, but not in cells harboring ΔyoaA. These results suggest that YoaA functions in conjunction with HolC.

In this work, we systematically discovered the basis of the synthetic lethality of four deletion mutations, Δnfo, ΔxthA, LD3-5-1 and LD20, using a series of reduced-genome strains. We also identified two multi-copy suppressors, holC and yoaA, using temperature-sensitive mutants and a chromosome library. Furthermore, we showed that the ΔxthA single mutant and the Δnfo ΔxthA double mutant became more sensitive to MMS upon addition of ΔyoaA. Thus, we revealed synthetic genetic interactions between YoaA and XthA related to survival from MMS exposure. A recent study reported a synthetic genetic interaction between YoaA and RadA, a RecA-related protein, involved in survival from azidothymidine (AZT) exposure (Cooper et al., 2015). Here, we describe additional synthetic genetic interactions between YoaA and HolC. YoaA is predicted to be a DNA helicase and may function together with DNA polymerase III. Alternatively, YoaA may form a complex with a free chi subunit and single-strand binding protein. Further biochemical analysis is needed to understand the mechanism by which YoaA is involved in DNA repair.

This study demonstrates that identification and analysis of synthetic lethality using reduced-genome strains should be a useful approach for elucidating many functional interactions.

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