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- Note -

Isolation of Temperature-sensitive Mutant *Escherichia coli* YrdC Involved in Universal t^{6}A tRNA Synthesis

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The YrdC superfamily is a group of proteins that are highly conserved in almost all organisms sequenced so far. YrdC in *Escherichia coli* was suggested to be involved in ribosome biogenesis, translation termination, cold adaptation, and threonylcarbamoyl adenosine formation in tRNA. In this study, to unambiguously demonstrate that *yrdC* is essential in *E. coli*, we constructed two *yrdC* mutant strains of *E. coli* and examined their phenotypes. In the temperature-sensitive *yrdC* mutant strain, cell growth stopped almost immediately under nonpermissive conditions and it appeared to accumulate 16S ribosomal RNA precursors without significant accumulation of 30S ribosomal subunits. We also cloned yeast and human homologs and demonstrated that they complement the *E. coli yrdC*-deletion strain. By mutational study, we demonstrated that the concave surface in the middle of the YrdC protein plays an important role in *E. coli*, yeast, and human versions. By comparison of two *yrdC*-deletion strains, we also unambiguously demonstrated that *yrdC* is essential for viability in *E. coli* and that the functions of its yeast and human homologs overlap with that of *E. coli* YrdC.

Key words : ATPase, *Escherichia coli*, threonylcarbamoyl adenosine, tRNA, YrdC

Introduction

The YrdC protein belongs to the superfamily of YrdC/YciO/Sua5 proteins, and it is ubiquitously found in both prokaryotes and eukaryotes [28]. The function of YrdC has been implicated in the formation of threonylcarbamoyl adenosine (t^{6}A) in tRNA [7, 13, 29]. However, its exact role in the process of tRNA modification has not been determined yet; nevertheless, its structural study indicated that *Escherichia coli* YrdC has double-stranded RNA-binding activity and its concave surface clustered with positive electrostatic potential appears to be critical for its dsRNA association [2, 9, 19, 28]. Another structural determination of *Sulfolobus tokodaii* Sua5 also revealed that it possesses nucleotide-binding activity in the concave area [19].

Previously, a genetic study showed that the *prfA1* allele encodes temperature-sensitive polypeptide Release Factor 1 and is associated with the enhanced translational misreading of UAG and UAA [26]. The temperature-sensitive phenotype of *prfA1* was also reported to be suppressed by deletion of the first 12 nucleotides of the *yrdC* open reading frame in *E. coli* [17]. Kaczanowska et al. suggested that the suppression by Δ*yrdC*12 (12 nucleotides deletion) is associated with impaired 30S ribosome biogenesis and showed marginal accumulation of 16S rRNA precursors without notable accumulation of 30S ribosomal subunits. Serial deletions of rRNA genes also suppressed the *prfA1* phenotype, implying that YrdC is a novel ribosome maturation factor. Moreover, the defective precursors of 16S rRNA in *prfA1* Δ*yrdC*12 (12 nucleotides deletion) cell lysates were fully processed to become normal 16S rRNA when purified YrdC proteins were added exogenously [18].

For yeast YrdC homolog, the *sua5-1* allele in *Saccharomyces cerevisiae* was identified as a suppressor of *cyc1-1019* mutant encoding an aberrant upstream AUG initiation codon followed by a stop codon, UAA. It was suggested that the *sua5-1* mutant is involved in translation, re-initiation, and/or mRNA stability. Sua5 null mutant exhibited a growth defect in minimal medium and a deficit in cytochrome *a,a*3 [11, 12].

Recently, Yacoubi et al. showed that the deletion of *sua5* in yeast led to the disappearance of t^{6}A at position 37 of tRNA, which in turn was recovered by the exogenous expression of *E. coli* YrdC [8]. Unlike in yeast, however, the modification defect associated with the deletion of *yrdC* in *E. coli* remains unknown [8], even though *E. coli* YrdC binds

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preferentially to partially modified $tRNA^{thr}$. Nevertheless, it has been demonstrated that t^6A is synthesized *in vitro* by the functional combination of YrdC, YgjD, YjeE, and YeaZ of *E. coli* [7]. Intriguingly, Meng et al. demonstrated that Sua5 binds to the core telomeric single-stranded DNA sequences in yeast and positively regulates telomere length through an association with telomere-binding proteins or telomeric DNA [22, 23]. They also demonstrated that mutations in the positively charged concave surface disrupt the telomeric DNA binding.

Unlike *E. coli* YrdC and yeast Sua5 proteins, ischemia/reperfusion-induced mouse YrdC (mYrdC/mIRIP, ischemia/reperfusion condition-induced protein), which associates with the membrane transporter regulator RS1 protein of mouse and human YrdC (hYrdC/hIRIP), was shown to interact with retinoblastoma-binding protein, RBBP10 [3, 16]. However, the functional association of human or mouse YrdC protein with tRNA modification or telomere DNA regulation is currently elusive. Thus, the defined role of YrdC is still controversial due to its divergent functional characterizations in different species, and as mentioned earlier, the tRNA modification defect was not observed by the deletion of *yrdC* in *E. coli* [8].

In this study, to demonstrate the essentiality of YrdC in *E. coli* and the functional complementation of eukaryotic homologs in the deletion of *yrdC*, we constructed *yrdC*-deletion and temperature-sensitive *yrdC* mutant strains and investigated their phenotypes. We also demonstrated that using the *yrdC*-deletion strain, the function of eukaryotic and bacterial homologs is likely conserved.

Materials and Methods

Growth conditions

Wild-type and *yrdC*-deletion strains were grown in Luria - Bertani (LB) medium. Table 1 summarizes the strains and plasmids used in this study. LB medium without arabinose, unless indicated otherwise, was routinely used for cell

Table 1. Bacterial strains and plasmids used in this study

Strains	Genotypes	References
BW25113	*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$	[6]
BY	*yrdC*::kan, pIEYrdC in BW25113	This study
Plasmids		
pIE	*lpp*^{p} *lac*^{po}, Amp^r, ori^{ts}	[15]
pBAD33	P_{BAD}, Cm^r	[10]
pIEYrdC	*yrdC*$^+$, pIE	[1]
pBADYrdC	*yrdC*$^+$, pBAD33	This study
pBADYrdCts	*yrdC* (Y25C, F95L), pBAD33	This study
pBADYrdC$_{Y25C}$	*yrdC* (Y25C), pBAD33	This study
pBADYrdC$_{F95L}$	*yrdC* (F95L), pBAD33	This study
pBADYrdC$_{T27L}$	*yrdC* (T27L), pBAD33	This study
pBADYrdC$_{K50I}$	*yrdC* (K50I), pBAD33	This study
pBADYrdC$_{L58A}$	*yrdC* (L58A), pBAD33	This study
pBADYrdC$_{R117A}$	*yrdC* (R117A), pBAD33	This study
pBADYrdC$_{L127A}$	*yrdC* (L127A), pBAD33	This study
pBADSua5	*sua5*$^+$, pBAD33	This study
pBADSua5$_{T70L}$	*sua5* (T70L), pBAD33	This study
pBADSua5$_{K93I}$	*sua5* (K93I), pBAD33	This study
pBADSua5$_{L101A}$	*sua5* (L101A), pBAD33	This study
pBADSua5$_{S107F}$	*sua5* (S107F), pBAD33	This study
pBADSua5$_{R174A}$	*sua5* (R174A), pBAD33	This study
pBADSua5$_{L184A}$	*sua5* (L184A), pBAD33	This study
pBADhYrdC	*hyrdC*$^+$, pBAD33	This study
pBADhYrdC$_{T87L}$	*hyrdC* (T87L), pBAD33	This study
pBADhYrdC$_{K110I}$	*hyrdC* (K110I), pBAD33	This study
pBADhYrdC$_{L118A}$	*hyrdC* (L118A), pBAD33	This study
pBADhYrdC$_{R173A}$	*hyrdC* (R173A), pBAD33	This study
pBADhYrdC$_{L183A}$	*hyrdC* (L183A), pBAD33	This study

growth, and antibiotics were used at concentrations of 50, 35, and 50 mg/ml for ampicillin, kanamycin, and chloramphenicol, respectively. Arabinose was added at a final concentration of 0.4% (w/v). Culture turbidity was measured at 600 nm.

Cloning and site-directed mutagenesis of *yrdC*

Primers were used to amplify the coding sequence of the wild-type *yrdC* from *E. coli*, human, and yeast. The polymerase chain reaction (PCR) products were digested with *Nde*I-*Hin*dIII; the digested DNA fragments were ligated into the *Nde*I-*Hin*dIII site of pBAD33 (an arabinose-inducible plasmid, Cm^r) [10]. The final clones are listed in Table 1. Site-directed mutagenesis was carried out by PCR and confirmed by sequencing analysis.

Construction of *yrdC*-deletion strain

Primers containing up- and downstream flanking sequences of *yrdC* were used to amplify the kanamycin cassette of pKD13 by PCR [6]. The strain BW25113 harboring pKD46 [γ β *exo* (λ Red recombinase), *bla,* ori^{ts}] [6] and a helper plasmid pBADYrdC (*yrdC*$^+$, pBAD33) was subjected to PCR- amplified linear DNA transformation, and the transformants resistant to chloramphenicol and kanamycin at 30°C were isolated. The gene disruption was verified by PCR. The resultant strain was used to prepare for P1 transduction [24]. The P1 lysate was transduced to the strain BW25113 harboring pIEYrdC, which is a helper plasmid containing *yrdC*, a temperature-sensitive replication origin, and an ampicillin- resistant gene [1]. The transductant resistant to ampicillin and kanamycin at 30°C was tested for temperature sensitivity to 42°C, yielding strain BY (*yrdC*-deletion strain). The deletion of *yrdC* in the BY strain was confirmed again by PCR.

Random mutagenesis and screening the temperature-sensitive mutants

Error-prone Taq polymerase was used for random mutagenesis of *yrdC* with dITP, as described by Lerner et al. [20] and Spee et al. [27]. The PCR products corresponding to the mutagenized open reading frame of *yrdC* were digested with *Nde*I-*Hin*dIII; the digested fragments were ligated into the *Nde*I-*Hin*dIII site of pBAD33; the ligation mixture was transformed into the BY cells. The clones were screened for temperature sensitivity at 42°C. The selected clones were retransformed into the BY cells and the temperature-sensitive phenotype of the transformants was confirmed. The plasmids consistently showing a growth defect at 42°C were sequenced.

Isolation of polysomes and sucrose density gradient sedimentation

Polysomes were prepared and resolved as described previously [15]. *E. coli* strains were grown at either 30°C or 42°C in 100 ml of LB medium to log phase. Upon reaching an appropriate culture density, polysomes were trapped by the addition of chloramphenicol to the culture at a final concentration of 0.1 mg/ml. After an additional 4 min of incubation, cells were harvested by centrifugation. The cell pellet was resuspended in 1 ml of buffer BP [20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NH_4Cl, and 5 mM β-mercaptoethanol]. Polysomes were resolved by applying 0.5 ml of the cell lysate to a 5%-40% linear sucrose density gradient (10 ml) in buffer BP with subsequent ultracentrifugation at 4°C in a Beckman SW41-Ti rotor for 2.5 hr at 150,000× g. The profile of polysomes was detected at 254 nm.

Results and Discussion

YrdC is essential in *E. coli*

Previously, it was suggested that YrdC may play a critical role in 30S ribosome biogenesis [5, 17], and a yeast homolog Sua5 was shown to be essential for normal cell growth [21]. Peculiarly, however, a combination of *prfA1* and Δ*yrdC*12 (first 12 nucleotides deleted) in *E. coli* was not detrimental [17], suggesting that the product of *yrdC* is not necessary in this specific genetic background. To unambiguously demonstrate that YrdC is pivotal for cell growth in *E. coli*, we attempted to delete the whole open reading frame of *yrdC* in wild-type *E. coli*. Wild-type cells were transformed with a helper plasmid, pIEYrdC, which contains a temperature-sensitive replication origin and an ampicillin-resistance gene. Subsequently, a kanamycin resistance cassette replaced the locus of *yrdC* in a cell harboring pIEYrdC. This conditional deletion of *yrdC* (strain BY) was confirmed by PCR using the transformants formed at 30°C on LB plates containing ampicillin and kanamycin. As shown in Fig. 1A, both wild-type and BY cells formed colonies at 30°C; however, BY cells formed smaller colonies on LB plates at 42°C. Subsequent streaking of these cells was found to be ampicillin-sensitive (data not shown) and did not lead to the colony formation even on LB plates without ampicillin at not

only 42°C but also 30°C (Fig. 1B). These results indicate that cells in these small colonies lost the helper plasmid, and that the deletion of *yrdC* in *E. coli* is detrimental to cell viability. This phenotype was further confirmed by measuring the generation time of BY cells grown in a liquid medium at a nonpermissive temperature, 42°C. The growth rate of BY cells decreased at 8 hr after an increase of temperature to 42°C, which corresponds to approximately 15 generations (Fig. 1C). Note that the helper plasmid in the BY strain was derived from a low-copy-number plasmid, pEL3 (~5 copies/chromosome at 30°C), whose replication is inhibited at 42°C [1, 4, 14]. Next, to verify the essentiality of *yrdC* and exclude the polar effect of gene deletion, the BY strain was transformed with pBAD33 or pBADYrdC. Cells transformed with empty vector pBAD33 formed small colonies, as consistently observed in Fig. 1A, and transformants of pBADYrdC recovered the normal growth at 42°C on LB plates even without an inducer, suggesting the essentiality of YrdC (Fig. 1D).

Both mouse and yeast YrdC homologs were proposed to be present at low abundance, suggesting that their expression in each organism is tightly regulated [16, 25]. This may be related to our observations shown in Fig. 1. The overexpression of YrdC from a helper plasmid enables cells to divide up to ~15 generations under nonpermissive conditions, resulting in the small colony formation on the first plating. Thus, it is tempting to speculate that *E. coli* YrdC is also a protein present in low abundance.

Isolation of temperature-sensitive YrdC

The BY cells exhibited an unexpected prolonged growth even under nonpermissive conditions (Fig. 1A, Fig. 1C), and the expression of YrdC from pBADYrdC in the absence of an inducer resulted in colony formation (Fig. 1D). Thus, it is likely that minimal plasmid-borne expression of YrdC is sufficient for viability. To circumvent this problem and to

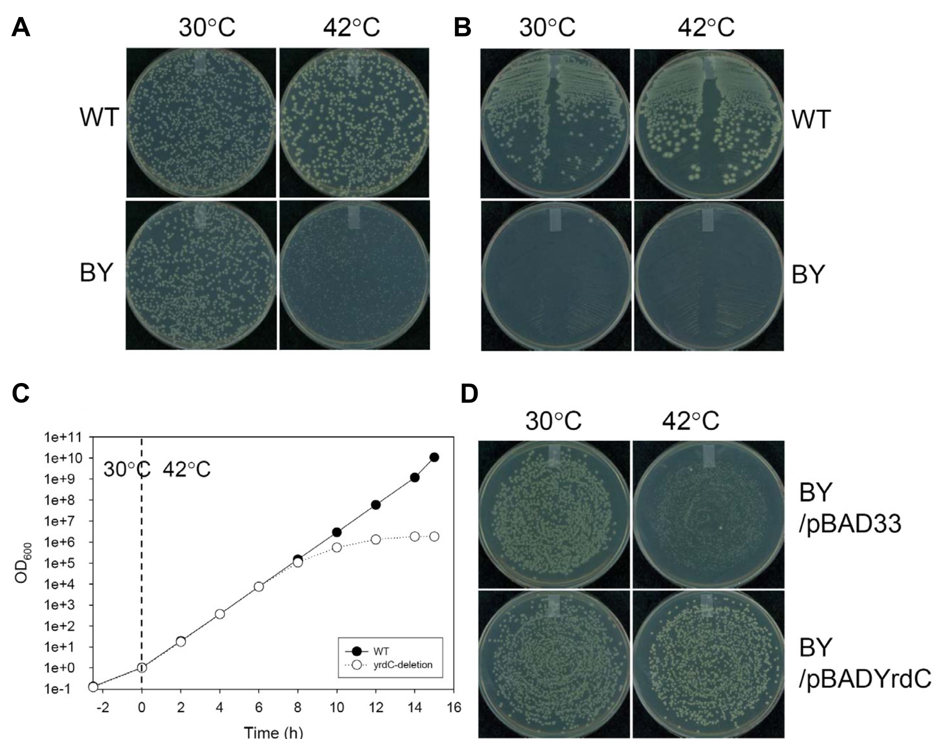

Fig. 1. YrdC is essential in *Escherichia coli*. (A) The wild-type and BY cells were grown overnight and plated on LB plates. For BY cells at 30°C, the plate contained ampicillin and kanamycin. Then, plates were incubated at 30°C or 42°C. (B) The respective cells from plates incubated at 42°C in (A) were restreaked on LB plates and incubated at 30°C or 42°C. (C) The wild-type and BY cells were grown at 30°C in LB for the wild-type strain or in LB medium containing ampicillin and kanamycin for the BY cells. Portions of both cultures were centrifuged to remove antibiotics and resuspended in prewarmed LB medium. The cultures were shifted to 42°C at the time indicated by a vertical line. During incubation at 42°C, the cultures were repeatedly diluted (1:20) into a freshly prewarmed medium. Closed circles, wild type; open circles, BY cells. (D) The BY cells were transformed with pBAD33 or pBADYrdC, and the transformants were incubated at 30°C and 42°C. For 30°C, the plates contained ampicillin and chloramphenicol, whereas for 42°C, they contained chloramphenicol, but not arabinose.

demonstrate the immediate lethal phenotype of *yrdC* deletion, *yrdC* was randomly mutagenized as described in Materials and Methods section to obtain a temperature-sensitive YrdC. The mutagenized *yrdC* fragments were ligated into pBAD33, and the ligation mixture was transformed into the BY cells. The clones were screened for a growth phenotype at 30°C and lethality at 42°C. The screening results revealed that the temperature-sensitive mutant YrdC expressed by the clone pBADYrdCts contains two substitutions at Tyr25 and Phe95 to Cys and Leu, respectively. These two mutations were separated by site-directed mutagenesis, yielding pBADYrdC$_{Y25C}$ and pBADYrdC$_{F95L}$, and were further examined to determine the residue responsible for the temperature sensitivity. BY cells were transformed with respective plasmids, and cells on the LB+Cm plate were first incubated at 42°C to remove the helper plasmid, pIEYrdC. The absence of the helper plasmid was subsequently confirmed by further incubation on LB plates containing ampicillin (data not shown). The colonies formed at 42°C were subsequently restreaked on the LB+Cm plates or M9+Cm minimal medium plates supplemented with 0.4% glucose, and the plates were incubated at 30°C or 42°C. As shown in Fig. 4A, either mutation could support the normal cell growth at 30°C; however, transformants of pBADYrdC$_{Y25C}$ appeared to form smaller colonies at 42°C than those of pBADYrdC$_{F95L}$. These results suggest that a combination of both Y25C and F95L mutations in YrdC caused the temperature sensitivity of growth. Next, we confirmed the temperature sensitivity by measuring the growth of cells harboring pBADYrdC, pBADYrdCts, or pBADYrdC$_{Y25C}$. These cells were grown first in LB medium containing chloramphenicol at 30°C for 2 hr; then, the cultures were transferred to 42°C for further incubation. The isogenic wild-type strain Δ*yrdC*/pBADYrdC grew exponentially in a serially diluted culture after an increase in temperature. By contrast, the growth rate of Δ*yrdC*/pBADYrdCts cells was more severely reduced at 42°C than that of Δ*yrdC*/pBADYrdC$_{Y25C}$ cells (Fig. 2B). Using Δ*yrdC* cells harboring pBADYrdC or pBADYrdCts, we analyzed the polysome profile to detect the abnormality of ribosome biogenesis. Isolation of polysomes and sucrose density gradient sedimentation were performed as described in Materials and Methods. No significant accumulation of ribosomal subunits was observed from the Δ*yrdC*/pBADYrdCts cells cultured at a non-permissive temperature (data not shown). Similarly, we did not observe the abnormal polysome profile of the BY cells grown at 42°C (data not shown). However, when we examined the total RNAs from Δ*yrdC*/pBADYrdCts cells grown at 42°C, the accumulation of 16S rRNA precursors was marginally detected at 2-6 hr after the increase in temperature, even without notable accumulation of 30S subunits (Fig. 2C).

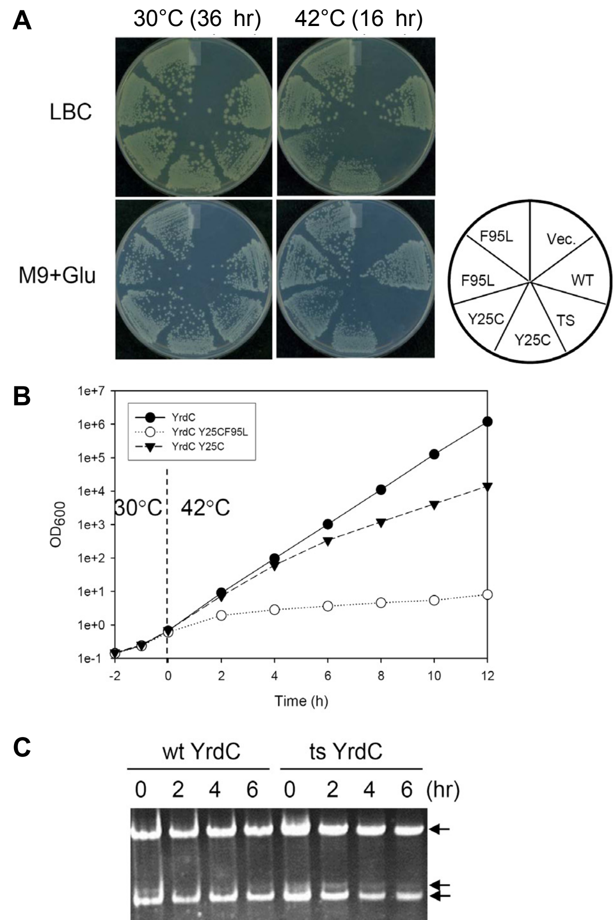

Fig. 2. Screening of temperature-sensitive YrdC protein and its phenotype. (A) Random mutagenesis of *yrdC* was carried out using dITP and $MnCl_2$, and temperature-sensitive YrdC was isolated as described in Materials and Methods. BY cells harboring pBAD33, pBADYrdC, pBADYrdCts, pBADYrdC$_{Y25C}$, or pBADYrdC$_{F95L}$ were streaked on LB or M9 plates supplemented with casamino acids. Both plates contained chloramphenicol and were incubated either at 30°C for 36 hr or at 42°C for 16 hr. (B) Temperature-sensitive phenotype of YrdCts-expressing cells. The Δ*yrdC* cells transformed with pBADYrdC, pBADYrdCts, or pBADYrdC$_{Y25C}$ were cultured in an LB medium containing chloramphenicol at 30°C, and further incubation was carried out as described in Fig. 1C. (C) Total RNAs of BY cells expressing wild-type or YrdCts. Aliquots of the cell culture in (B) were taken at 0, 2, 4, and 6 hr after an increase in temperature and were used to extract total RNAs. Here, 1.5 μg of total RNA for each sample was applied on a 3.5% polyacrylamide gel. Arrows indicate 23S, 17S, and 16S rRNA from the top.

Consistent with this, the deletion of *yrdC* (12 nucleotides deletion) in the *prfA1* genetic background did not cause substantial accumulation of 30S; nevertheless, rRNA processing was impaired, leading to the accumulation of 17S rRNA [17]. Furthermore, yeast Sua5 plays roles in translation initiation and 40S ribosome assembly [12, 21, 25]. However, we could not detect the direct interaction between the 30S ribosome subunit and YrdC (data not shown). Nevertheless, our results suggest that YrdCts cause improper processing of 16S rRNA, implying a ribosome biogenesis factor. However, we do not rule out the possibility that defective tRNA modification in *yrdC* null indirectly causes abnormal rRNA biosynthesis [8, 13, 30].

Eukaryotic homologs of YrdC complement *E. coli* *yrdC*-deletion strain

Not only do human (aa residues 63-209) and yeast (aa residues 53-222) YrdC domains share a sequence homology of 44% and 47% with *E. coli* YrdC, respectively, but the structures of *E. coli* YrdC and Sua5 proteins are also superimposable with a positively charged concave area and ATP-binding domain [19, 28]. Therefore, to investigate whether the expression of human and yeast YrdC homologs in *E. coli* complements the BY cells, we cloned human *yrdC* and yeast *sua5* in pBAD33, yielding pBADhYrdC and pBADSua5. The plasmids pBAD33, pBADYrdC, pBADhYrdC, and pBADSua5 were transformed into the BY cells, and the transformed cells were first plated on LB plates containing ampicillin and chloramphenicol at 30°C. Colonies from conditions of 30°C were further streaked on LB plates containing chloramphenicol in the presence or absence of 0.4% arabinose, and the plates were then incubated at 42°C overnight (Fig. 3A). Colonies were then picked and restreaked on the same plates. Next, the plates were incubated at 42°C. As shown in Fig. 3B, in the absence of arabinose, only cells harboring pBADYrdC formed colonies at 42°C; however, Δ*yrdC* cells were able to form colonies when human and yeast proteins were overexpressed in the presence of 0.4% arabinose. As mentioned previously, defective t^6A modification in yeast *sua5* mutant was reversed by the expression of *E. coli* YrdC; thus, our results suggest that the function of *E. coli*, human, and yeast YrdC proteins is conserved and that they are mutually functional in each organism.

Mutational analyses of *E. coli*, yeast, and human YrdC proteins

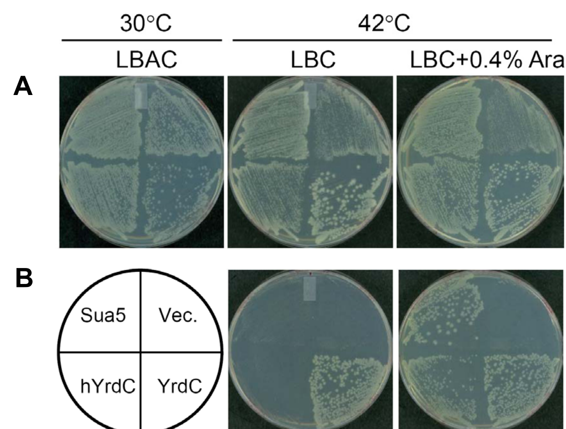

Fig. 3. Complementation of the BY strain by human and yeast YrdC homologs. (A) The BY cells were transformed with the plasmids as indicated in (B) and incubated first at 30°C on LB plates containing ampicillin and chloramphenicol. Then, each transformant was restreaked and incubated at 42°C on LB plates containing chloramphenicol in the presence or absence of 0.4% arabinose. (B) The colonies formed at 42°C from (A) were restreaked on the same plates at 42°C as in (A), followed by second incubation at 42°C.

Previously, it was shown that a double mutant [T87L (corresponding to T27 in *E. coli* YrdC) and K110I (K50 in *E. coli* YrdC)] of human YrdC exhibited a dominant-negative phenotype by enhancing MPP^+ transport activity [16]. In yeast Sua5, S107F mutation in the YrdC domain was shown to eliminate its function [21]. More recently, *Sulfolobus tokodaii* Sua5 protein was proposed to bind nucleotide, AMP (or ATP) [19], and the highly conserved residues Leu58, Arg117, and Leu127 (numbering in *E. coli* YrdC) were proposed to be involved in nucleotide binding. Interestingly, all of these residues are located in the concave area and have been implicated in double-stranded RNA binding. Hence, we attempted to analyze these residues described earlier to determine their functional importance in YrdC homologs. For this purpose, we performed site-directed mutagenesis using pBADYrdC, pBADSua5, and pBADhYrdC. Each mutation was verified by sequencing. Plasmids were first transformed into the BY cells, and the complementation experiment was performed as shown in Fig. 3. In the absence of arabinose, the *E. coli* YrdC protein carrying L58A or L127A mutation was able to support cell growth, whereas the YrdC protein carrying T27L, K50I, or R117A mutation failed to do so. Note that in the presence of 0.4% arabinose, proteins carrying the mutation T27L or K50I complemented the *yrdC*-deletion cells, suggesting that these mutant proteins are partially

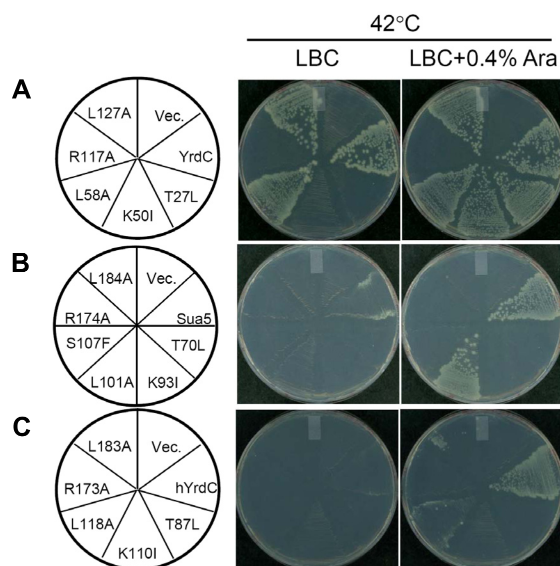

Fig. 4. The mutational analyses of human, yeast, and *Escherichia coli* YrdC proteins. Five mutant clones for *E. coli* YrdC, six for yeast Sua5, and five for human YrdC (hYrdC) were constructed via site-directed PCR mutagenesis. The resulting plasmids were transformed into the BY cells, and the complementation experiment was carried out as described in Fig. 3. The plates shown here are from the second incubation at 42°C. The LB plates contained either chloramphenicol or chloramphenicol and 0.4% arabinose. (A) YrdC and its mutants; (B) Sua5 and its mutants; and (C) hYrdC and its mutants.

functional (Fig. 4A). Corresponding mutations were introduced in yeast and human YrdC homologs, and the plasmids carrying the resulting constructs were transformed into the BY cells. As shown in Fig. 3B and Fig. 3C, none of the human and yeast mutants except yeast $Sua5_{L101A}$ could complement the Δ*yrdC* cells, suggesting that the concave surface of the YrdC protein composed of highly conserved residues is critical for its RNA association and/or ATP binding.

In summary, our study suggested that the YrdC homologs play a conserved role in ribosome biogenesis and/or tRNA modification. In addition, it suggested that the temperature-sensitive mutant YrdC isolated in this study may provide a platform to investigate the biosynthetic process of t^6A tRNA modification by *E. coli* YrdC, which has not been elucidated yet, probably due to the unclear phenotype associated with the deletion of *yrdC*.

Acknowledgments

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초록 : 대장균에서 t^6A tRNA의 생합성에 관여하는 필수 단백질 YrdC의 온도 민감형 돌연변이 분리

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YrdC 수퍼 패밀리는 지금까지 유전 서열이 알려진 거의 모든 생명체에서 매우 잘 보존 된 단백질 중 하나이다. *Escherichia coli*의 YrdC는 리보솜 생합성, 번역 종결, 저온 적응, tRNA에서 threonylcarbamoyl adenosine의 형성에 관여하는 것으로 제안되었다. 이 연구에서, *yrdC* 유전자가 대장균에서 필수적이라는 것을 명확하게 증명하기 위해, 대장균에서 두 개의 *yrdC* 결손 돌연변이 균주를 만들고 그 표현형을 조사하였다. 특히 온도에 민감한 *yrdC* 돌연변이 균주는 42°C 온도 조건 하에서 거의 즉시 세포 성장을 멈추었으며 30S 리보솜 단위체의 상당한 축적없이 16S rRNA 전구체를 축적하는 것으로 나타났다. 또한 효모와 인간의 *yrdC* 유전자를 클로닝하여 이들이 대장균 *yrdC* 결손 균주의 성장억제를 회복 할 수 있다는 것을 입증하였다. 이밖에도 여러 돌연변이 연구에 의해, 우리는 YrdC 단백질의 중간에 위치한 오목한 표면이 대장균, 효모 및 인간의 YrdC 단백질에서 중요한 역할을 한다는 것을 보여주었다. 따라서, 두 개의 *yrdC* 결손 균주를 비교하여, *yrdC* 유전자가 대장균에서 생존력에 필수적이며, 효모 및 인간 동족체의 기능이 대장균 YrdC의 기능과 중복된다는 것을 규명하였고, 이 균주를 이용하여 아직까지 밝혀지지 않은 대장균 YrdC 단백질이 tRNA 형성에 관여한다는 것을 증명할 수 있는 토대를 제공한다는데 의의가 있다.