EsrE–A yigP Locus-Encoded Transcript—Is a 3′ UTR sRNA Involved in the Respiratory Chain of E. coli

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The yigP locus is widely conserved among γ-proteobacteria. Mutation of the yigP locus impacts aerobic growth of Gram-negative bacteria. However, the underlying mechanism of how the yigP locus influences aerobic growth remains largely unknown. Here, we demonstrated that the yigP locus in Escherichia coli encodes two transcripts; the mRNA of ubiquinone biosynthesis protein, UbiJ, and the 3′ untranslated region small regulatory RNA (sRNA), EsrE. EsrE is an independent transcript that is transcribed using an internal promoter of the yigP locus. Surprisingly, we found that both the EsrE sRNA and UbiJ protein were required for Q₈ biosynthesis, and were sufficient to rescue the growth defect ascribed to deletion of the yigP locus. Moreover, our data showed that EsrE targeted multiple mRNAs involved in several cellular processes including murein biosynthesis and the tricarboxylic acid cycle. Among these targets, sdhD mRNA that encodes one subunit of succinate dehydrogenase (SDH), was significantly activated. Our findings provided an insight into the important function of EsrE in bacterial adaptation to various environments, as well as coordinating different aspects of bacterial physiology.

Keywords: yigP locus, aerobic growth, Q₈, EsrE sRNA, succinate dehydrogenase, E. coli

INTRODUCTION

Gene regulation, which occurs at multiple levels including transcriptional and post-translational control, is a vital mechanism across all domains of life (Bobrovskyy and Vanderpool, 2013). One emerging class of post-transcriptional regulators in bacteria is small regulatory RNAs (sRNAs; Bobrovskyy and Vanderpool, 2013). Previous studies suggest that sRNAs are typically short transcripts that vary from 50 to 500 nucleotides and rarely contain an open reading frame (ORF; Bobrovskyy and Vanderpool, 2013). sRNAs act by perfect or limited base-pairing to target mRNAs to influence their stability or/and translation (Updegrove et al., 2015). In γ-proteobacteria, most sRNAs require the RNA chaperone, Hfq, to stabilize themselves and promote sRNA–mRNA intermolecular interaction (Chao and Vogel, 2010; Vogel and Luisi, 2011). Such interaction...
principally inhibits the translation and/or induces the degradation of their target mRNAs in a ribonuclease E (RNase E)-dependent manner (Masse and Gottesman, 2002; Masse et al., 2003; Morita et al., 2005; Frohlich and Vogel, 2009). However, compared to their negative effect on gene expression, the positive regulation by sRNAs remains largely unknown (Papenfort and Vanderpool, 2015). Besides the most characterized intergenic sRNAs, recent RNA-seq studies suggest bacterial sRNA-encoding genes are also located in coding regions (Lalaouna et al., 2015; Miyakoshi et al., 2015b). Therefore, these 3′ untranslated region (UTR) sRNAs belong to a newly characterized non-intergenic class. They are produced by either mRNA processing or transcription from independent promoters within genes. In both cases, the 3′ UTR sRNAs share the same sequences with the 3′ regions of the coding genes (Miyakoshi et al., 2015b). Therefore, these 3′ UTR sRNAs pose a challenge for ongoing gene or locus function characterization.

All 11 ubi genes (ubiA–J and ubiX) are required for the biosynthesis of Q8 in Escherichia coli. Most of their products catalyze specific reactions in the biosynthetic process, starting from chorismate (Van Beilen and Hellingwerf, 2016). The yigP (renamed ubiF) locus is highly conserved among almost 80 organisms, many of which are γ-proteobacteria. In E. coli, yigP is located between ubiE and ubib loci, and may be synchronously transcribed with them under an upstream promoter of ubiE (Poon et al., 2000; Aussel et al., 2014a). UbiE is well-known for its C methylation reactions in both Q8 and menaquinone (MK8) biosynthesis (Aussel et al., 2014a). A mammalian homolog of UbiB, COQ8A (ADCK3), was recently found to display ATPase activity and interacted with lipid CoQ intermediates (Stefely et al., 2016). However, though yigP mutant strains of Salmonella and E. coli showed significant defects in Q8 biosynthesis (Aussel et al., 2014a), the underlying mechanism remains unrevealed.

Our previous studies found that the 3′ region of yigP transcribed an sRNA named EsrE with indispensable function in E. coli (Chen et al., 2012). A recent study in Salmonella found that the yigP locus encoded a protein named UbiJ required for Q8 biosynthesis. Based on evidence that a “scrambled mutation” of the 3′ region of Salmonella ubiJ had no phenotype, it was concluded that the aerobic growth-promoting function of the yigP locus was mediated by UbiJ but not the sRNA, EsrE (Aussel et al., 2014a). In this work, we provide new evidence that the yigP locus contains two genes, ubij and esrE. UbiJ encoded a protein, UbiJ, while esrE transcribed an sRNA, EsrE. Furthermore, our results showed that besides UbiJ, the sRNA EsrE was also critical for the aerobic growth of E. coli and required for Q8 biosynthesis. In addition, we found that EsrE might maintain cellular processes of E. coli by activating multiple mRNAs.

**MATERIALS AND METHODS**

**Bacterial Strains and Growth Conditions**
The wild-type is E. coli K-12 strain JM83. Unless otherwise stated, the cells were grown at 37°C in liquid or on solid Luria-Bertani (LB) media supplemented with chloramphenicol (30 μg/mL) or ampicillin (100 μg/mL) as indicated. All strains and plasmids used here are listed in Supplementary Table S1.

**Inactivation of the yigP Locus**
The λ Red-mediated recombination method was used to delete different regions of the yigP locus with an insert of apramycin resistance cassette. The mutant fragments were transferred to E. coli JM83 by electroporation.

**Construction of a 3′ Flag-yigP-Fused E. coli Strain**
To carry out an E. coli strain that expresses a fused YigP protein with a C-terminal [Gly5,Ser3] linker plus a triple-Flag tag (DYKDHDGDYKDHDIDYKDDDDK), a pMAK705-derived temperature-sensitive plasmid, pMAK-yigP-flag, was created using an approach described previously (Bush et al., 2013). Using the pMAK-yigP-flag according to previous methods (Chen et al., 2012), the chromosomal yigP insertion strain, WT::flag, was constructed.

**Quinone Extraction and Analysis**
The extraction and analysis of quinones was performed as described by Ozier et al. (2015) and Van Beilen and Hellingwerf (2016). The cells were cultured for 10 h before collection by centrifugation, washed twice with distilled water, and their wet weights determined. A total of 150 μL of 0.15 M KCl (a Q8 solution used as an internal standard, 100 μg/mL in ethyl alcohol, 0.863 μL/mg of wet weight) and 0.6 mL of methanol were added to cell pellets, and the tubes vortexed for 15 min. Then 0.4 mL of petroleum ether (boiling range, 40–60°C) was added and the tubes vortexed for 5 min. The phases were separated by centrifugation at 5000 rpm at room temperature for 5 min. The upper petroleum ether layer was transferred to a fresh tube. Petroleum ether (0.4 mL) was added to the methanol-containing tube, and the extraction was repeated once more. The petroleum ether layers were combined and dried. The lipids were resuspended in 100 μL of ethyl alcohol.

The samples were fractionated with high-performance liquid chromatography (HPLC) using a reversed-phase C18 column (Betabasic-18, 5 μm, 4.6 mm × 250 mm, Agilent Technologies). The column was equilibrated with pure ethyl alcohol as the mobile phase at a flow rate of 1 mL/min. Detection of quinones was performed using an UV/Vis absorption detector at 275 nm for ubiquinone (UQ).

**Real-time qRT-PCR**
Isolation of bacterial total RNA was performed with High Pure RNA Isolation Kit (Roche Diagnostics, Germany) according to the manufacturer’s instructions. Total RNA was the template for qRT-PCR reaction using Reverse Transcription M-MLV (RNase H-) kit (TaKaRa, Japan) and SYBR Green PCR Master Mix (TOYOBO, Japan). The primers for qRT-PCR are listed in Supplementary Table S2. The levels of gene expressions were normalized using the rpoD transcript data.
SDS-PAGE and Western Blot Analysis

To investigate whether the yigP locus encodes a protein, SDS-PAGE and western blotting were performed. All the strains were grown to an indicated time in LB media, and the cells were washed with phosphate-buffered saline. The samples were sonicated and total protein quantified following standard protocols. The protein was denatured at 100°C for 5 min. Total protein from each sample was loaded for SDS-PAGE, and western blotting was performed with Flag-tag primary and horseradish-peroxidase-conjugated goat anti-mouse secondary (AOGMA, United States) antibodies.

β-Galactosidase Assays

Cells were cultured overnight and then subcultured at 1:100 in fresh LB. After growing for 5 h to an optical density (OD) of 2, equivalent cell densities were collected, pelleted, and permeabilized by ultrasonication. Ortho-nitrophenyl-β-D-galactopyranoside hydrolysis was determined in triplicate to measure β-galactosidase activity as described previously (Wang et al., 2012).

RESULTS

The yigP Locus Encodes Two Products: EsrE and UbiJ

The ubiE, yigP, and ubiB loci in E. coli are considered an operon (Poon et al., 2000) essential for Q8 biosynthesis (Aussel et al., 2014b). However, how yigP contributes to this process is still unclear. In our previous study, we found that the 3′ region of yigP locus encoded a small RNA called EsrE that might have essential functions in E. coli (Chen et al., 2012). Recently, the yigP locus was reported to encode a protein called UbiJ that is required for Q8 biosynthesis and aerobic growth in Salmonella and E. coli (Aussel et al., 2014a). To further determine whether yigP locus has two transcripts, we designed two pairs of primers, yigP-S1/2 and yigP-X1/2 targeting its 5′ and 3′ regions, respectively, for qRT-PCR analysis (Figure 1A). Because ubiE, yigP, and ubiB loci are considered an operon (Poon et al., 2000), ubiE and ubiB mRNAs were also examined (Figure 1B). As shown by qRT-PCR, ubiJ mRNA was expressed throughout the lifetime of the bacteria (Figure 1B). In contrast, esrE expression was only detected during the late exponential growth phase (approximately 5 h) as indicated by the finding that the transcript from the 3′ region was more abundant than the transcript from the 5′ region at 5 h (Figure 1B). These observations suggested that UbiJ was the constitutively expressed product of the yigP locus, while EsrE was transiently generated during the transition to the stationary phase.

In order to determine the contribution of the two transcripts to aerobic growth, we constructed three deletion mutants containing different regions of the E. coli yigP locus (Figure 2A). All mutants were successfully obtained. As shown in Supplementary Figure S1, the colonies of the full region (∆yigP) and 3′ region (∆yigP-3′end) mutant strains that grew on LB plates under aerobic conditions were much smaller than the colonies of the 5′ region mutant strain (∆yigP-5′end) (Supplementary Figure S1). These mutant strains and the wild type strain were then cultured in LB under aerobic conditions. Consistent with our previous results, ∆yigP and ∆yigP-3′end strains exhibited significant growth defects compared to the wild type strain. In contrast, ∆yigP-5′end strain grew just as well as the wild type strain (Figure 2B). Because the yigP locus was truncated in ∆yigP-5′end strain to prevent UbiJ translation in vivo, our results suggest EsrE was the product of the yigP locus that maintained the aerobic growth of E. coli.

To further determine the functional region of the yigP locus, we transformed two plasmids containing different truncated fragments (Chen et al., 2012) in proximity to the esrE-coding region into the ΔyigP strain (Figure 2C). The plasmid carrying the 367 bp whole esrE-coding sequence fragment partially (P4P2) restored the growth defect of ΔyigP strain, whereas the 267 bp partial esrE-coding sequence fragment (P43L9) failed to do so (Figure 2D). Because the PlacZ promoter located in pUC18 had been removed, the experiment also revealed that the 3′ region fragment (P4P2) had its own promoter. Hence, the EsrE transcript from an independent promoter located within the yigP locus contributed to the growth of E. coli.

In addition, the yigP locus was previously shown to be essential for Q8 biosynthesis, and the amount of Q8 was tightly related to the aerobic growth of E. coli (Aussel et al., 2014a). We therefore measured the cellular Q8 content of the three mutant (Figure 2A) and the wild type strains by HPLC analysis (Figure 2E) (Ozeir et al., 2015; Van Beilen and Hellingwerf, 2016). Unlike the growth
phenotype (Figure 2B), the Q₈ content in ΔyigP-3′end strain was 50% of the wild type strain (33 and 67 pmol, respectively) (Figure 2F). Reasoning that ΔyigP-3′end strain had no UbiJ protein expression in vivo, EsrE was responsible for the half amount of Q₈, which might be enough to maintain normal aerobic growth of E. coli (Figure 2B; Aussel et al., 2014a,b). Meanwhile, the ΔyigP and ΔyigP-3′end strains had tiny amounts of Q₈ (Figure 2F), which was in agreement with previous evidence that the yigP locus was required for the synthesis of Q₈. Taken together, these results indicated that both EsrE and UbiJ from the yigP locus were required for Q₈ production in E. coli.

**EsrE Is Required for Aerobic Growth of E. coli as an sRNA**

We investigated whether the 3′ region of the yigP locus encodes a polypeptide or only EsrE as an sRNA. A 3 × Flag plus Linker coding sequence (111 bp) was chromosomally fused via homologous recombination to the 3′ end of the yigP locus in wild type strain using the temperature-sensitive plasmid pMAK-yigP-flag, yielding the WT::flag strain (Figure 3A). The identified quinones, Q₈ and Q₁₀, are indicated. (F) Quantification of cellular Q₈ content (n = 3) of the same cells as in panel (A) in picomoles per milligram of wet weight. Error bars represent standard deviation.
FIGURE 3 | The yigP locus encodes EsrE and a 23-kDa protein, UbiJ, both of which have important effects on the growth of E. coli. (A) Schematic representation of mutant strain, which Linker plus 3 × Flag tag sequences were introduced before the stop codon of the yigP locus in wild type bacteria, named WT::flag. In addition, Linker plus 3 × Flag tag sequences were introduced before the stop codon of different yigP fragments in plasmid pUC18 to yield pUC-yigP-flag, pUC-P4P2-flag, and pUC-P43P2-flag. YigP means the full-length fragment (including 241 bp sequences upstream yigP locus, considered as the promoter region of ubiJ), P4P2 (367 bp) indicates larger region of esrE-coding sequence than P43P2, while P43P2 (313 bp) contains the minimal region of esrE-coding sequence. (B) Western blot analysis of WT::flag and wild type bacteria with pMAK-yigP-flag. The yigP variants were separated by 15% SDS-PAGE, blotted onto PVDF membranes and hybridized with a Flag tag antibody or RNAP-α antibody. RNAP-α means RNA polymerase α subunit protein used as a loading control. (C) ΔyigP (filled circles), ΔyigP/pUC-yigP-flag (filled inverted triangles), ΔyigP/pUC-P4P2-flag (filled squares), ΔyigP/pUC-P43P2-flag (filled triangles), and pUC18 (filled rhombuses) were grown overnight and then diluted to an OD_{600} of 0.03 in LB under aerobic conditions. Growth was monitored at 600 nm. The experiment was performed at least three times, and identical patterns were obtained. (D) HPLC separation of the strains described for panel (C), except ΔyigP/pUC18 with the eluate analyzed with A_{275}. The identified quinones, Q_{8} and Q_{10}, are indicated. (E) Quantification of cellular Q_{8} content (n = 3) of the same cells as in panel (C), except ΔyigP/pUC18 in picomoles per milligram of wet weight. Error bars represent standard deviation. (F) Western blot analysis of pUC-yigP-flag, pUC-P4P2-flag, and pUC-P43P2-flag in ΔyigP strain. The yigP variants were separated by 15% SDS-PAGE, blotted onto PVDF membranes and hybridized with a Flag tag antibody or RNAP-α antibody. RNAP-α means RNA polymerase α subunit protein used as a loading control.
the RNA, EsrE, and the other was the 23 kDa protein, UbiJ. Collectively, our results showed that there were two function of the 3′ (Supplementary Figure S2B). These results suggested that the esrE all three plasmids transcribed Figure 3F present any band by immunoblotting (protein, while both pUC-P4P2-flag and pUC-P43P2-flag did not biosynthesis. Similarly, only pUC-yigP-flag encoded a 26 kDa protein, while both pUC-P4P2-flag and pUC-P43P2-flag did not present any band by immunoblotting (Figure 3F). Meanwhile, all three plasmids transcribed esrE as determined by qRT-PCR (Supplementary Figure S2B). These results suggested that the function of the 3′ region fragment relied on an sRNA instead of a polypeptide. Collectively, our results showed that there were two products encoded by different regions of the yigP locus: one was the RNA, EsrE, and the other was the 23 kDa protein, UbiJ.

To further examine the role of EsrE, we employed four different P43P2 frameshift mutant fragments (Figure 4A). The ΔyigP strain was transformed with four plasmids, named pT-002, pT-003, pT-004, and pT-005 (Chen et al., 2012) that contained these mutant fragments. All plasmids significantly complemented the impaired aerobic growth of ΔyigP strain when cultured in LB (Figure 4B). It is worth mentioning that the ΔyigP strain with pT-005 was much smaller than the others when grown on LB plates for up to 24 h, but then reached similar size after 40 h of culture (Figure 4C). This indicated that the deletion of site 005 (adenine) might have partly affected the rescue capability of the P43P2 fragment. Meanwhile, we also checked the cellular Q8 content of the four strains. All four strains harbored enough amounts of Q8 (Figure 4D) to recover the growth defect. Overall, because the four mutation sites could affect all potential ORFs, their rescue capabilities provided strong evidence that all the potential ORFs were immune to frameshift mutations, even though these mutant fragments could not recover the growth defect to the same level as the P43P2 fragment (Supplementary Figure S3). Taken together, these data showed that the esrE gene located in the 3′ region of the yigP locus encoded a 3′ UTR sRNA required for *E. coli* aerobic growth and biosynthesis of Q8.

**EsrE Targets Multiple mRNAs In Vivo**

To uncover the physiological role of EsrE, we tried to search its regulons. Because most sRNAs target mRNAs to regulate their expression levels via perfect or limited base-pairing, the potential regulons of EsrE were screened with two comparative prediction
FIGURE 5 | Experimental validation of EsrE target predictions. (A) Fluorescence of wild type bacteria carrying the indicated gfp fusions and EsrE plasmid (pT-P4P2) or control plasmid (pT-NS). The bacteria were grown in triplicate in LB liquid culture and measured at the indicated growth phase (at 5 h, about OD$_{600}$ of 2). The fluorescence values were given in arbitrary units and corrected for basal fluorescence of a wild type strain harboring plasmid pXG-0. (B) Relative activity of β-galactosidase in wild type bacteria carrying the indicated lacZ fusions and EsrE (pT-P4P2) or control (pT-NS) plasmid. The bacteria were grown in triplicate in LB liquid culture and were collected at the indicated growth phase (at 5 h, about OD$_{600}$ of 2). β-Galactosidase assays were then performed according to a protocol described previously (Wang et al., 2012). ***P < 0.001.

FIGURE 6 | EsrE is required for succinate dehydrogenase activity. (A) Succinate dehydrogenase (SDH) activity assays were carried out on wild type, ΔyigP, and ΔyigP strains transformed with pUC-P4P2-flag at four different growth phases (5, 7.5, 10, and 22 h). Equivalent cell densities were collected, pelleted, and permeabilized by ultrasonication. The measurement of SDH activity was performed in triplicate using the Succinate Dehydrogenase assay kit (Nanjing Jiancheng Bioengineering Institute, China). The experiment was performed at least three times, and identical patterns were obtained. (B) Wild type, ΔyigP, and ΔyigP strains transformed with pUC-P4P2-flag were grown overnight in LB and then diluted to an OD$_{600}$ of 0.03 in 100 mL of 0.2% minimal glucose or 0.4% minimal sodium succinate medium. Growth was monitored at 600 nm. The ordinate is the OD$_{600}$ ratio. The experiment was performed at least three times, and identical patterns were obtained. The results of a representative experiment are shown. *P < 0.05, **P < 0.01.

algorithms for sRNA targets, CopraRNA (Wright et al., 2014) and TargetRNA (Kery et al., 2014). Twenty-four candidates were chosen for validation in vivo (Supplementary Table S3).

To further verify these candidates, translational fusions were generated by integrating the 5′ UTRs in-frame with the first codon of gfp or lacZ (the fluorescence of some GFP fusions are not high enough for detection) and downstream of the heterologous P$_{lcrO}$ promoter (Supplementary Table S3) (Urban and Vogel, 2007). Wild type cells were then transformed with these fusion plasmids. GFP fluorescence or β-galactosidase activity was monitored in the presence of EsrE overexpression or control plasmids. Among all the candidates, four showed...
**DISCUSSION**

**The yigP Locus Encodes Two Products: EsrE and UbiJ**

UQ is a redox-active lipid that is widely distributed in nature. The UQ structure has a conserved aromatic ring and an isoprenoid side chain of various lengths. The length of isoprenyl chain varies among species; there are six in *Saccharomyces cerevisiae*, eight in *E. coli*, and 10 in humans (Turunen et al., 2004; Bentinger et al., 2010; Nowicka and Kruk, 2010). Therefore, UQ in *E. coli* is designated Q₈. The Q₈ biosynthetic pathway is highly conserved and requires almost 11 genes, named *ubi* (Meganathan, 2001; Tran and Clarke, 2007). Q₈ acts as a redox carrier in the plasma membrane, plays vital roles in aerobic respiration, oxidative stress adaptation, regulation of gene expression and other pathways dependent on the proton motive force (Aussel et al., 2014b). Recently, the *yigP* locus was renamed *ubiJ* that was characterized as an important element for Q₈ biosynthesis in *Salmonella* and *E. coli* under aerobic conditions (Aussel et al., 2014a). Aussel et al. (2014a) believed that the aerobic growth defect was attributed to the blockage of Q₈ biosynthesis due to mutant *yigP* locus. No sRNA was produced from the *yigP* locus because their frameshift mutant fragments at the C-terminal (the last 50 amino acids) coding sequence failed to rescue the growth defect (Aussel et al., 2014a). Though our results also showed that the *yigP* locus was required for Q₈ biosynthesis in *E. coli*, we found that EsrE sRNA was also required for this process because in the absence of UbiJ, Q₈ could still be produced in *E. coli* (Figures 2–4). Based on the evidence that the UbiJ deficient (frameshift) strain showed no phenotype and EsrE could only partially rescue the defect caused by 3′ UTR deletion at the *yigP* locus in aerobic growth, we inferred that not all the cellular Q₈ content were required for aerobic growth of *E. coli*. Some minimal level (threshold level) of Q₈ could be enough to maintain the high growth rate. This then led us to question whether the functions of EsrE and UbiJ were tightly related, and if there were advantages of hosting *esrE* within *ubiJ*. Our data showed that besides promoting Q₈ biosynthesis, EsrE was also required for SDH activity, which suggests that the function of EsrE could be tied to UbiJ, and the two cooperated with each other in the respiratory chain of *E. coli*. However, more investigations are needed to verify this hypothesis.

**A New Insight into Gene Annotation: 3′ UTR-Embedded sRNAs**

Ongoing studies reveal that sRNA genes are not limited to intergenic regions, as they are also detected in coding regions (Lalaouna et al., 2015; Miyakoshi et al., 2015a). These sRNAs might pose a problem for gene annotation because there can be more than one functional product from a single gene locus. One emerging class of such sRNAs is 3′ UTR sRNAs. Miyakoshi et al. (2015b) proposed that 3′ UTR sRNAs could be divided into two basic types according to their biogenesis. Type 1 sRNAs are transcribed from independent promoters within the coding region or the 3′ UTR of known genes. This type includes MicL (Guo et al., 2014), EsrE (described here) in *E. coli*, and DapZ in...
**FIGURE 7** Proposed function of the yigP locus. The yigP locus may have two products, both of which are required for the aerobic growth of *E. coli*. One is a protein encoded by *ubiJ* that functions as a new factor involved in Q₈ biosynthesis under aerobiosis. The other is sRNA transcribed from the 3′ region of the yigP locus and also required for Q₈ biosynthesis as well as interacting with multiple targets involved in different cellular processes such as murein biosynthesis and the TCA cycle. This sRNA especially activates *sdhD* mRNA.

*Salmonella enterica* (Chao et al., 2012). Type II 3′ UTR sRNAs such as CpxQ (Chao and Vogel, 2016) and SorC in *S. enterica* (Miyakoshi et al., 2015a) strictly originate from the processing of the parental mRNAs. 3′ UTR sRNAs usually share partial sequences with their respective mRNA loci (Chao et al., 2012; Guo et al., 2014; Miyakoshi et al., 2015b; Chao and Vogel, 2016). Thus the phenotypes attributed to these loci may not depend solely on the protein. The functions of both products need to be considered. Moreover, the correlation between 3′ UTR sRNAs and their parental mRNAs are currently ambiguous since only a few examples have been studied. DapZ and MicL functions have been proven to have no relation with their parental mRNAs, while SorC and CpxQ mediate cross talk between their respective mRNAs (Chao et al., 2012; Guo et al., 2014; Miyakoshi et al., 2015a; Chao and Vogel, 2016). It seems that Type II sRNAs are more related to their parental mRNAs than Type I. Here we found that EsrE, a Type I sRNA, has four targets. Among them, three (*murE, murF, and sdhD*) were activated and one (*argI*) was repressed by EsrE (Figures 5A,B). These targets are functionally unrelated, which makes EsrE function more diversified thereby requiring more efforts to uncover. Besides, EsrE is important for cellular Q₈ biosynthesis, but the corresponding targets and regulatory mechanisms are still unknown. Additionally, EsrE could activate *sdhD* to increase the levels of SDH, which connects the TCA cycle with the ETC. Thus, we anticipate that EsrE might have a vital role in regulating the respiratory chain of *E. coli*. Meanwhile, its respective mRNA, *ubiJ*, is also a key element of the aerobic ETC of *E. coli* (Aussel et al., 2014a). In general, Type I sRNA could cooperate with their parental mRNA. But still, the correlation of the parental mRNA function and targets of 3′ UTR sRNAs remain to be elucidated.

In summary, our study provided new evidence that the yigP locus had two products, the UbiJ protein and EsrE sRNA. Both products were essential for Q₈ biosynthesis and further contributed to aerobic growth of *E. coli* (Figure 7). Our results also showed that EsrE might target multiple mRNAs, including *murE, murF, sdhD*, and *argI*, which are involved in different cellular processes. Furthermore, our data indicated EsrE was required for the activity of SDH in succinate-dependent growth of *E. coli*. Additionally, our characterization of EsrE indicated that sRNAs (sRNAs from UTRs) may be masked in protein-coding regions. Phenotypes generally caused by deficiencies in protein may also be due to uncovered sRNA defects.

**AUTHOR CONTRIBUTIONS**

HW and HZ conceived and designed the experiments. HX, XY, and QT performed the experiments. JY contributed reagents/materials/analysis tools. HX, HW, and HZ analyzed and wrote the paper.

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SUPPLEMENTARY MATERIAL

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