An efficient design strategy for a whole-cell biosensor based on engineered ribosome binding sequences

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Abstract In prokaryotes, the ribosome binding sequence (RBS), located in the 5′ untranslated region (5′ UTR) of an mRNA, plays a critical role in enhancing mRNA translation and stability. To evaluate the effect of the RBS on the sensitivity and signal intensity of an environmental whole-cell biosensor, three *Escherichia coli*-based biosensors that respond to benzene, toluene, ethylbenzene, and the xylenes (BTEX) were constructed; the three biosensors have the same *Pu* promoter and *xylR* regulator from the *Pseudomonas putida* TOL plasmid but differ in the engineered RBS in their reporter genes. The results from time and dose-dependent induction of luminescence activity by 2-chlorotoluene showed that the BTEX-SE and BTEX-SD biosensors with engineered RBS had signal intensities approximately 10–35 times higher than the primary BTEX-W biosensor. The limits of detection (LOD) of the BTEX-SE and BTEX-SD biosensors were also significantly lower than the LOD of the BTEX-W biosensor ($20 \pm 5 \mu\text{mol L}^{-1}$ and $25 \pm 5 \mu\text{mol L}^{-1}$ vs. $120 \pm 10 \mu\text{mol L}^{-1}$). Moreover, the BTEX-SE and BTEX-SD biosensors responded three times more rapidly to the analytes. These results suggest that rationally designed RBS in the 5′ UTR of a reporter gene may be a promising strategy for increasing the sensitivity, signal intensity, and response speed of whole-cell biosensors.

Keywords Whole-cell biosensor · Ribosome binding sequence · Signal intensity · Detection sensitivity

Introduction

Our environment is being polluted with an increasing number of industrial and domestic pollutants, resulting in the contamination of water, atmosphere, and soil. Therefore, it is essential to develop rapid and efficient methods to evaluate the environmental risks of pollutants, especially for their biotoxicity and bioavailability. Because of their low cost, ability to detect bioavailability, and ease-of-use, whole-cell biosensors have been developed and used widely as simple and practical approaches to the detection of a variety of agents, including heavy metals, toxic cellular organics, and metabolic products [1, 2]. Moreover, biosensors can be customized with dimensions small enough to monitor the dispersal of analytes in the microenvironment [3, 4]. However, despite these advantages, and with the exception of heavy metals, whole-cell biosensors can efficiently detect most analytes only at micromolar concentrations. Moreover, low signal intensities hinder detection when fluorescent proteins and single-copy reporter systems are used for in-situ assessment of bioavailability [5, 6]. Therefore, efforts should be taken to overcome these limitations for the practical application of whole-cell biosensors.

Traditionally, the strategy for construction of a whole-cell biosensor is based on a specific network of transcriptional regulation. In this network, a particular ligand binds a transcription factor, which in turn alters its affinity toward specific DNA sequences, resulting in differential access of RNA polymerase to the promoter and differential expression of mRNA [7, 8]. On the basis of this process, many successful efforts in the past few decades, for example

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engineered promoters, directed evolution of regulatory proteins, and reporter options, have improved the sensitivity and signal intensity of whole-cell biosensors [9–11].

In prokaryotes, the 5′ untranslated region (5′ UTR) of an mRNA has a significant effect on translational efficiency and mRNA stability by directing the initiation codon to the P site of the small ribosomal subunit and by masking endonuclease recognition sites in mRNAs [12–14]. It is clear that two major motifs in the 5′ UTRs of bacterial mRNAs, the Shine–Dalgarno (SD) sequence and translational enhancers, are directly involved in initiation of translation [15–18]. The SD sequence affects translational efficiency through its base-pairing with the anti-SD sequence of the 16S ribosomal RNA [19, 20]. In addition, a translational enhancer, usually an A/U-rich motif located further upstream in the 5′ UTR, can also improve mRNA translational efficiency by interacting with the S1 protein of the ribosome [21]. A recent study showed that among 162 completed prokaryotic genomes the number of the SD-directed genes varied from 11.6% to 90.8%, which implies that the populations of non-SD-directed genes (i.e., those not containing a SD-like sequence in their 5′ UTRs) are significant, although the translation initiation mechanism for these mRNAs is unclear [14]. However, there are no reports about whether an RBS can improve the sensitivity and the signal intensity of a whole-cell biosensor or improve a strategy based on the translational level of a whole-cell biosensor.

In this study, based on typical RBS signatures, three types of whole-cell biosensor with the same transcriptional regulation unit but different engineered RBS were designed and tested. The results clearly demonstrate that the RBS has a significant effect on the sensitivity and signal intensity of a whole-cell biosensor. The results also suggest that the rational design of a reporter gene 5′ UTR may be a promising strategy for increasing the sensitivity and signal intensity of whole-cell biosensors.

Materials and methods

Construction of the biosensor plasmids

Plasmid DNA was isolated using the BAC/PAC DNA isolation system (Omega Bio-Tec, USA). When necessary, DNA fragments were purified from agarose gels using the Omega Bio-Tec (USA) gel extraction kit. General DNA purification was performed with the Omega Bio-Tec Cycle Pure kit. All restriction endonucleases, the high-fidelity thermophilic DNA polymerase (Vent DNA polymerase) and T4 ligase were obtained from NEB (USA). DNA

oligonucleotide primers were synthesized by Invitrogen (Beijing Office, China). Nucleotide sequences were determined using an Applied Biosystems Model 377 Prism DNA sequencer (Augct, Beijing, China).

The *Pu* promoter was amplified from the TOL plasmid of *Pseudomonas putida* ATCC 33015 (ATCC, USA) using the polymerase chain reaction (PCR) and primers P₁ (5′-ATTAGCGGCCGCCCCGGGAAAGCGCGATGAAC-3′) and P₂ (5′-ACTGGGATCCTCACAGACTCCAGGCGTAACG-3′) containing *NotI* and *BamHI* restriction enzyme digestion sites (shown in bold italics). Purified PCR products and the pGEM-*luc* plasmid were digested with *NotI* and *BamHI* and ligated to create the plasmid pGP. The *xylR* gene was amplified with primers P₃ (5′-CGATCTCGAGATTTAATGTGGGCTGCTTGGT-3′) and P₄ (5′-GTACGAGCTCTTTTCACACAACCTGGGGCG-3′) and inserted into the *XhoI* and *SacI* sites of pGP to create the plasmid pGPX (Table 1).

Because the pGEM-*luc* vector is a *lacZ* inserted plasmid, to eliminate the *lacZ* promoter and the *lacZ* gene in pGEM-*luc* vector, primers P₅ (5′-AATTGCGGCCGCGTGCACCTGCAGGCATGCTCTAGAGGAAATTGTAAGCGTTAATAT-3′) and P₆ (5′-GACTGAGCTCTCTTCCGCTTCCTCGCTC-3′) containing *NotI* and *SacI* digestion sites were used to amplify the fragment containing ori, Amp^r, and fl ori from the pGEM-*luc* plasmid. After *NotI* and *SacI* digestion, this fragment was ligated to the cassette from the pGPX plasmid to form the pBTEX plasmid.

To prevent possible ribosome skipping [20], the *luc* gene of pBTEX was amplified using primers P₇ (5′-GGGCCCCGGATCCAAATGGAAGA-3′) containing a *BamHI* site and primers P₈ (5′-CGATCTCGAGCTATCAT-TACAATTTGGACTTTCCGCCC-3′) containing three termination codons (underlined) and an *XhoI* site and replaced by this PCR fragment to construct pBTEX-W [1, 20].

To construct plasmids with a modified RBS, the *luc* gene of pBTEX was replaced by a *luc* fragment amplified with primers P₉ (5′-ACTGGGATCCAAAGGAGATATACATATGGAAGACGCCAAAAACATAAAG3′), containing the RBS sequence (underlined) from the T7 phage *g10* gene [22], and P₈ to obtain plasmid pBTEX-SD. The *luc* gene of pBTEX was replaced by the same method with P₁₀ (5′-ACTGGGATCCTTAACTTTAAGAAGGAGATATACATATGGAAGACGCCAAAAACATAAAG3′), containing the same SD sequence plus an enhancer (underlined) located near the 5′ end and P₈ to obtain plasmid pBTEX-SE. DNA sequencing and restriction digest analyses of the fragments verified the proper sequences. The three plasmids pBTEX-W, pBTEX-SD, and pBTEX-SE were transformed into *Escherichia coli* DH5α (Tiangen Biotech, China) to create the corresponding whole-cell biosensors.

Table 1 Strains and plasmids used in this study

Strains or plasmids	Relevant characteristics	Reference/origin
<i>Escherichia coli</i> DH5 α	F ⁻ ϕ 80lacZ Δ M15 Δ (lacZYA-argF) U169 deoR recA1 endA1 hsdR17 (r _k ⁻ , mk ⁺) phoA supE44 λ ⁻ thi-1 gyrA96 relA1.	Tiagen Biotech (Peking, China)
<i>Pseudomonas putida</i> ATCC33015	Tol*, BETX*	ATCC
pGEM- <i>luc</i>	<i>luc</i> gene, Amp ^r , pUC replicon, <i>luc</i> :: <i>lacZ</i>	Promega
pGP	<i>luc</i> , <i>Pu</i> from Tol, Amp ^r pUC replicon, <i>luc</i> :: <i>lacZ</i>	This study
pGPX	<i>luc</i> , <i>xylR</i> and <i>Pu</i> from Tol, Amp ^r , pUC replicon, <i>luc</i> :: <i>lacZ</i>	This study
pBTEx	<i>luc</i> , <i>xylR</i> and <i>Pu</i> from Tol, Amp ^r , pUC replicon	This study
pBTEx-W	<i>luc</i> , <i>xylR</i> and <i>Pu</i> from Tol, Amp ^r , pUC replicon	This study
pBTEx-SD	<i>luc</i> with SD, <i>xylR</i> and <i>Pu</i> from Tol, Amp ^r , pUC replicon	This study
pBTEx-SE	<i>luc</i> with SD and enhancer, <i>xylR</i> and <i>Pu</i> from Tol, Amp ^r , pUC replicon	This study

All strains and plasmids used in this study are shown in this table

Tol* and BETX* denote the TOL plasmid and the ability to grow on culture medium with benzene, toluene, ethylbenzene, and the xylenes as carbon source. Amp^r denotes resistance to ampicillin

Induction of luciferase by 2-chlorotoluene

Single colonies of *E. coli* DH5 α cells harboring the corresponding biosensor plasmids were grown in 50 mL Luria–Bertani (LB) medium containing 100 μ mol L⁻¹ ampicillin at 37 °C. Cells were left to grow in log phase (8 to 12 h) to an optical density at 600 nm (OD₆₀₀) of 1.2. After this growth period, the cell culture was diluted with LB medium to an OD₆₀₀ of 0.4, and luciferase expression was induced by mixing 5 mL diluted culture with 5 mL LB medium containing increasing amounts of 2-chlorotoluene dissolved in DMSO as described by Ikeno et al. [23]. Induction was performed in 100-mL glass vials followed by incubation at 23 °C and 250 rpm for 30 min, and the culture was cooled on ice for 10 min before luciferase activity was detected. Luciferase activity was measured using the E1500 luciferase assay system and Glomax–Muti microplate multimode reader (Promega, USA) in accordance with the supplier's instructions.

RNA preparation

RNA was extracted from cells grown in the presence or absence of 250 μ mol L⁻¹ 2-chlorotoluene using the SV Total RNA isolation system (Promega). RNA samples were eluted with DEPC-treated water (Takara, Japan), and OD_{260/280} measurements were obtained by use of a Nano Drop 2000 spectrophotometer (Thermo, USA). Samples with OD_{260/280} ratios between 1.8 and 2.1 and concentrations ~300 ng μ L⁻¹ were used for subsequent analyses.

cDNA synthesis

For quantitative PCR analysis of RNA, cDNA synthesis was performed using the M-MLV reverse transcriptase (RT; Promega). Briefly, 1 μ g RNA template was mixed with random hexamer primers in less than 15 μ L water, heated to 70 °C and then immediately cooled on ice. Next, 5 μ L M-MLV 5 $\times$ reaction buffer, 1.25 μ L dNTP mixture (10 mmol L⁻¹ each), 25 units recombinant RNasin ribonuclease inhibitor (Promega), and 200 units M-MLV RT were added with nuclease-free water (Takara) to a final volume of 25 μ L. The reaction was incubated for 60 min at 37 °C.

Quantitative real-time polymerase chain reaction (qRT-PCR)

A housekeeping gene, *rpoS*, was used as an internal standard to calculate the relative quantity of each *luc* reporter gene mRNA [24]. The levels of *rpoS* mRNA with induction and the *luc* mRNA with and without induction were calculated relative to that of *rpoS* without induction.

The qRT-PCR reactions were performed in triplicate using the MX3005P real-time PCR system (Stratagene, USA) and GoTaq qPCR Master Mix (Promega). Primers (P₁₁, 5' GAGATACGCCCTGGTTCCTG3' and P₁₂, 5' TGCATACGACGATTCTGTGATT3' for *luc*. P₁₃, 5' TAACCTGCGTCTGGTGGTAAAA3' and P₁₄, 5' CGTATGTTGAAGACGGAAACC3' for *rpoS*) were obtained from Invitrogen (USA). The qRT-PCR reactions were performed in a 25- μ L reaction with 0.2 mmol L⁻¹ primer. The reaction mixture was denatured at 95 °C for 2 min and subjected to 40

cycles of the following thermal conditions: 95 °C (15 s), 57 °C (30 s), and 72 °C (30 s). All values reported are means from three independent experiments.

Methods used in data analysis

To evaluate detection sensitivity, the signal-to-noise ratio (SNR) was defined as the signal value of an effector-exposed sample divided by the signal value of its no-effector control. The limit of detection (LOD) was calculated as described elsewhere [25].

Data from three separate experiments were normalized. Origin version 7.5 was used to generate nonlinear least-squares fits of the data to the Hill equation:

$$y = y_{\max} - \{(y_{\max} - y_{\min}) / (1 + K_{1/2} \times [2 - \text{chlorotoluene}]^{1/n})\}$$

as described by Willardson and colleagues [1]. In this equation, y is the luminescence at a given concentration of 2-chlorotoluene, $y_{\max}$ is the maximum luminescence at the saturating concentration of 2-chlorotoluene, and $y_{\min}$ is the luminescence at a 2-chlorotoluene concentration of zero. $K_{1/2}$ is the concentration of 2-chlorotoluene at which the half-maximum effect is observed, and n is the Hill coefficient, n_{app} .

The relative amounts of *rpoS* mRNA and the *luc* gene mRNAs were quantitated by qRT-PCR using the method reported by Schmittgen [26].

Results and discussion

Construction of plasmids with different RBS in whole-cell biosensors

We constructed three plasmids, pBTEx-W, pBTEx-SD, and pBTEx-SE, each designed to express a firefly luciferase gene in response to benzene, toluene, ethylbenzene, and the xylenes (BTEx). Each plasmid contained the same BTEx-response elements from the *Pseudomonas putida* TOL plasmid, but they differed in the RBS for the *luc* reporter gene (Fig. 1). As shown in Fig. 1a, the primary plasmid pBTEx-W, which lacked a canonical SD sequence in the 5' UTR, was constructed as described elsewhere [1]. The *Pu* promoter was inserted just upstream of the *luc* gene between the *Bam*HI and *Not*I restriction sites. The promoter fragment spans 333 bp of the TOL plasmid *Pu* region, including the upstream regulatory sequences for XylR binding, the *ntr/nif*-24 and 12 promoter sequences, and the transcription initiation site. The *xylR* regulatory unit was placed directly downstream of the *luc* gene in the same orientation as *luc* between the *Xho*I and *Sac*I sites. The SD sequence (AGGAG) from the T7 phage *g10* gene was

introduced into the RBS site of the *luc* gene in pBTEx-W to create pBTEx-SD (Fig. 1b). A nine-base enhancer sequence, TTAAC TTTA, was introduced upstream of the RBS in BTEx-SD to create the plasmid BTEx-SE (Fig. 1c). When *E. coli* DH5 α cells were transformed with these plasmids, consistent luminescence signals in response to 2-chlorotoluene could be detected.

Dose-dependent induction of luminescence activity with 2-chlorotoluene

The dose-dependent response of the biosensors to 2-chlorotoluene at 30 min had a sigmoidal dependence on 2-chlorotoluene concentration, and the data fit the Hill equation well (Fig. 2). A nonlinear least-squares fit of the data also gave different $K_{1/2}$ values, which represent the concentrations of 2-chlorotoluene required for half-maximum signal intensity. The BTEx-W biosensor had a $K_{1/2}$ of $114.6 \pm 7.6 \mu\text{mol L}^{-1}$ (mean $\pm$ standard deviation), higher than the $K_{1/2}$ of BTEx-SE and BTEx-SD, which were $88.1 \pm 5.1 \mu\text{mol L}^{-1}$ and $48 \pm 3.3 \mu\text{mol L}^{-1}$, respectively. The signal intensity of the BTEx-W biosensor increased slowly during the course of detection. Specifically,

Fig. 1 Plasmid map and schematic diagram of the reporter plasmids. Important features of the BTEx series of biosensors are indicated, including the location and orientation of the *Pu* promoter, the *luc* gene, and the *xylR* transcriptional activator gene. a, b, and c show the 5' UTR sequences of the *luc* gene of biosensors BTEx-W, BTEx-SD, and BTEx-SE, respectively. The SD sequence of the biosensors and the A/U-rich enhancer of the BTEx-SE biosensor are shown by rectangular frames and denoted in bold face. The translational initiation codon ATG is also in bold face

Fig. 2 The 2-chlorotoluene concentration dependence of biosensor cell luminescence. Shown are measurements of the luminescence from biosensors BTEX-W (**a**), BTEX-SD (**b**), and BTEX-SE (**c**) induced by different concentrations of 2-chlorotoluene. The error bars represent the standard deviations of three replicates of each sample within the same experiment. Curves represent the nonlinear least-squares fit of the data to the Hill equation

there was no apparent increase at concentrations below $60 \mu\text{mol L}^{-1}$ (Fig. 2a). In contrast, the signal intensities of the other two biosensors, especially BTEX-SD, increased rapidly before the maximum signal intensity was reached (Fig. 2b, c). In addition, the slopes of the Hill equation for the three biosensors correlated well with the SNR results. Interestingly, the BTEX-SE biosensor achieved the highest signal intensity, but the BTEX-SD biosensor had the highest SNR and the highest slope among the three biosensors. To understand this phenomenon, the mRNA expression and mRNA stability for each biosensor should be investigated further. Nevertheless, there are differences between the BTEX-SD and BTEX-SE biosensors in performance; the

LOD values of both biosensors with an SD sequence or an SD sequence plus enhancer sequence were lower than that of BTEX-W ($25 \pm 5 \mu\text{mol L}^{-1}$, $20 \pm 5 \mu\text{mol L}^{-1}$ vs. $120 \pm 10 \mu\text{mol L}^{-1}$ at 30 min, Fig. 2). These results and the aforementioned observations indicate that the RBS contained in the 5' UTR of a reporter gene has significant effects on the sensitivity and signal intensity of the whole-cell biosensors.

Time-dependent induction of luciferase activity with 2-chlorotoluene

Figure 3 shows the time courses of signal intensities in *E. coli* DH5 α cells harboring the three different plasmids with or without induction by $250 \mu\text{mol L}^{-1}$ 2-chlorotoluene. On induction, the signal intensities expressed by BTEX-SD and BTEX-SE reached 1.93×10^6 U and 6.43×10^6 U, respectively, at 120 min (means of three replicates); these values were approximately 10 and 35 times higher than the signal from the primary biosensor BTEX-W. These results are consistent with the known ability of SD sequences and A/U-rich translational enhancers to increase translational efficiency up to 100 times by serving as high-affinity binding sites for the ribosome [15, 16, 27]. In the absence of 2-chlorotoluene, the signal intensities also increased over the same period as a result of cell growth, but the pattern was obviously different among the three whole-cell biosensors. Compared with the extremely weak SNR of BTEX-W, which was 0.98 ± 0.02 at 5 min and 1.5 ± 0.02 at 120 min (Fig. 3c), the SNR of BTEX-SD and BTEX-SE rapidly reached 3.17 ± 0.08 and 1.62 ± 0.05 at 5 min, and further increased to 10.25 ± 0.27 and 4.45 ± 0.05 at 120 min. Therefore, unlike the primary biosensor BTEX-W, which only marginally distinguished the SNR after 15 min, the BTEX-SD and BTEX-SE biosensors achieved much higher SNR values within 5 min. Even at lower concentrations of effector ($50 \mu\text{mol L}^{-1}$ 2-chlorotoluene), both the BTEX-SD and BTEX-SE biosensors performed well within 5 min; in contrast, the BTEX-W biosensor was completely unable to detect the analytes within 5 min (data not shown). These results indicated that the signals from the biosensors with either an SD sequence or an SD sequence plus a translational enhancer can readily distinguish the induced signal from the un-induced control by achieving high signal intensity and reduced detection time, properties which are quite useful for in-situ assessments of bioavailability.

mRNA quantitation

To calculate the relative amount of mRNA from each *luc* reporter gene, we used a housekeeping gene, *rpoS*, as an internal standard. The amount of *rpoS* mRNA in the biosensor-containing cells subjected to induction was almost the same as the amount of *rpoS* mRNA in cells

Fig. 3 Comparison of the luminescence and SNR of the three whole-cell biosensors during the course of 2-chlorotoluene induction. Shown are the time-dependent luciferase expression measurements and SNR values from BTEX-W (filled circles, empty circles), BTEX-SD (filled triangles, empty triangles) and BTEX-SE (filled diamonds, empty diamonds) plasmids. The *solid symbols* represent induced samples and their SNR whereas the *open symbols* represent the un-induced samples. Panel **a** shows the induced and un-induced luminescence activity of the BTEX-W, BTEX-SD and BTEX-SE biosensors. The *inset* in panel **a** shows the signal intensity of the BTEX-W biosensor, which was considerably weaker than for the BTEX-SD and BTEX-SE biosensors. Panel **b** shows the SNR of the three biosensors

without induction (Fig. 4). This result confirms that transcription of *rpoS* is unaffected by growth conditions and is suitable for use as an internal standard.

The relative amount of *luc* mRNA induced from the BTEX-SD biosensor was 29.1 ± 5.1 , which was about three times as much as the *luc* mRNA derived from BTEX-W and BTEX-SE, which were 9.8 ± 2.1 and 12.3 ± 1.7 , respectively (Fig. 4). There were no significant differences between the relative amounts of *luc* mRNA without induction; the amounts were 6.8 ± 1.0 for BTEX-W, 8.7 ± 1.2 for BTEX-SD, and 7.7 ± 1.7 for BTEX-SE (Fig. 4).

The relative amount of induced BTEX-SD *luc* mRNA was three times higher than the mRNA from BTEX-W. However, this finding does not mean that the transcriptional efficiency was improved by insertion of the SD sequence, because no transcriptional motifs were modified. The result is likely to be because the BTEX-SD *luc* mRNA is more

stable as a result of the SD insertion. In *E. coli*, mRNA degradation is a complex process that includes an initial, rate-limiting endonucleolytic cleavage (usually mediated by RNase E) followed by 3'-5' exonucleolytic degradation of the cleavage products [28–30]. Endonucleolytic attacks can be blocked to some extent by increased translational efficiency because, at higher rates of translation, ribosomes occlude a greater number of endonuclease recognition sites [12, 13]. Therefore, it is likely that the SD-mediated increased translational efficiency of the BTEX-SD *luc* mRNA led to increased stability of the mRNA.

Although the BTEX-SD and BTEX-SE biosensors shared the same SD sequence, they did not express the same amounts of *luc* mRNA. This difference may be because the AU-rich translational enhancer in BTEX-SE promoted rapid decay of its mRNA. When located within 5' UTRs, the A/AU rich motifs can serve as translational enhancers by interacting with ribosomal protein S1, an essential component of the *E. coli* translational machinery [13, 30, 31]. However, AU-rich single stranded regions have also been shown to be targets for RNase E, thereby bringing about rapid mRNA decay [32–35]. Thus, RNase E susceptibility induced by the A/U-rich motif may explain why the BTEX-SE and BTEX-SD biosensors produced different amounts of mRNA despite sharing the same SD sequence.

Our results show that the BTEX-SE biosensor has the highest signal intensity and lowest amount of *luc* mRNA; thus, the BTEX-SE reporter mRNA has the highest translational efficiency. Although a high translational effi-

Fig. 4 Relative amounts of mRNA. qRT-PCR was used to quantitate the mRNA levels of the *rpoS* housekeeping gene and the *luc* gene expressed in the presence and absence of the 2-chlorotoluene inducer ($250 \mu\text{mol L}^{-1}$). The transcription levels of *rpoS* under the induced condition and the *luc* gene with or without induction were calculated relative to that of *rpoS* without induction. The *columns* in **a** indicate the relative quantification value of mRNA from the *rpoS* gene. The value was approximately unity, which confirmed that transcription of *rpoS* is unaffected by growth condition. The *columns* in **b** and **c** filled with *transverse lines*, *oblique lines*, and *vertical lines* represent the relative amounts of *luc* mRNA from BTEX-W, BTEX-SD, and BTEX-SE with and without induction, respectively

ciency can stabilize an mRNA, the A/U-rich translational enhancer did not stabilize mRNA in our study. Thus, there may be competition between RNase E and the ribosomes. The internal portions of mRNA bound by ribosomes will be protected from RNase E-mediated endonuclease cleavage. However, other parts of the mRNA, e.g., the 5' UTR and the translation initiation site, are not protected by ribosomes and can be cleaved readily by RNase E. Thus, the A/U-rich element in our study probably acted as both a translational enhancer and an RNase E target.

Our results also show that different RBS motifs can affect the sensitivity and the signal intensity of a whole-cell biosensor or affect a strategy based on the translational level of a whole-cell biosensor. Other factors, for example the spacing between the SD sequence and the initiation codon, different initiation codons, mRNA stability, and translational termination, can affect the expression efficiency of the corresponding gene [20, 36–38]. All of these motifs may also affect the responses of whole-cell biosensors. Combined with these effective methods, improved whole cell biosensors with better sensitivity might be suitable for evaluating the environmental risks of the pollutants in situ.

Conclusions

The results presented here indicate that engineered 5' UTRs of a reporter gene have dramatic effects on both the signal intensity and the sensitivity of whole-cell biosensors; thus, modification at the transcriptional level and systematic modification at the translational level are effective in improving the response characteristics of a whole-cell biosensor. Although *E. coli* was used as the host cell in this study, similar SD-like RBS and A/AU-rich enhancer motifs have been found to act as translational enhancing elements in many other bacteria [15, 39–41]. Therefore, the strategy reported here may be applicable to other bacterial-based biosensors.

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