Control of Novel Energy Production by Anaerobic Respiration Using Citrate via a Citrate-Responsive Two-Component System

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Research objective

The aim of this study was to understand the mechanisms of citrate utilization and response in *Escherichia coli*, including the genomic transcriptional regulation of the citrate-responsive two-component regulatory system, CitAB, and its physiological role. *E. coli* strain K-12 cannot grow on citrate as a single carbon source, but can use it as a substrate for a type of metabolism known as “citrate fermentation,” which utilizes part of the tricarboxylic acid cycle in a reductive direction to consume reduction forces produced from metabolism, such as glycolysis under anaerobic conditions. This type of metabolism has been reported to be transcriptionally regulated by the two-component system, CitAB (CitA is the histidine kinase and CitB is the response regulator), which responds to extracellular citrate under anaerobic conditions. However, the role of CitAB in regulating the whole genome is unknown. Therefore, we aimed to identify the genomic transcriptional regulatory network of CitB in *E. coli* and determine its physiological role.

Methods

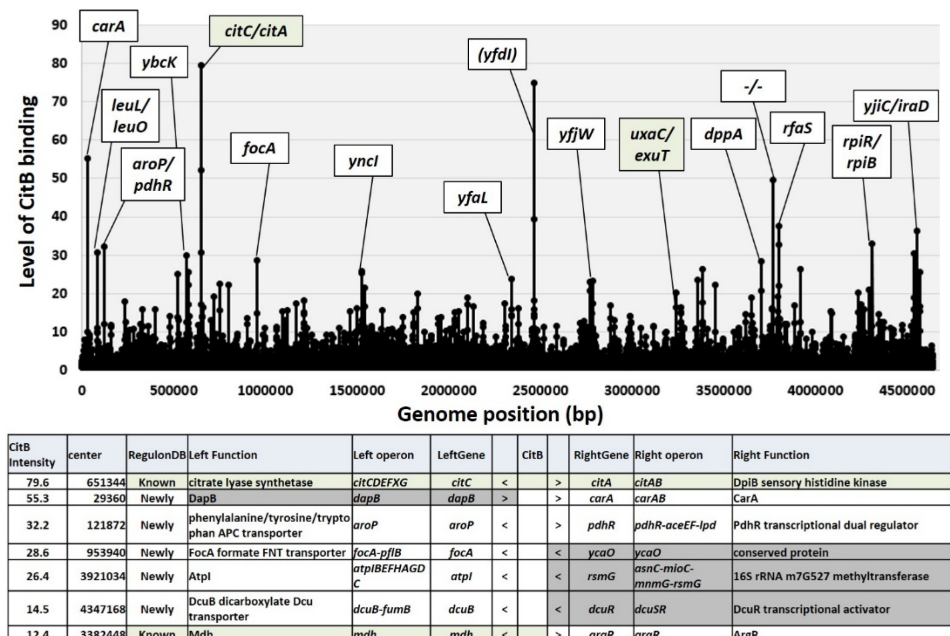
Transcriptional regulatory networks form a hierarchical structure. Therefore, to understand the essential regulatory mechanisms of transcriptional control, it is necessary to identify direct regulatory targets that are distinct from indirect effects. In addition, the presence of other factors in the cell causes competitive inhibition of the genome, and the tested transcription factors are not always expressed or activated *in vivo*. In this study, we comprehensively identified the genomic binding regions of CitB using a novel Genomic SELEX (gSELEX) method. gSELEX is an *in vitro* method in which purified transcription factors are mixed with genomic DNA fragments, and the DNA sequences of the complexes formed are analyzed. The gSELEX method enables the comprehensive identification of direct binding sequences in the genome[1)]. This method has the advantage that direct genomic target sequences can be obtained, even for transcription factors whose expression or activation conditions are unknown, and it is not affected by the presence of other competitive factors in the cell. In this study, CitB target sequences obtained using the gSELEX method were analyzed using tiling arrays. The binding activity of the CitB target was confirmed using gel shift assays.

To validate the regulation of the newly identified CitB target genes, wild-type and *citAB*-deficient *E. coli* strains were cultured in glucose-containing medium under anaerobic conditions, and mRNA levels with and without citrate (20 mM) addition were compared using reverse transcription-quantitative polymerase chain reaction. Next, citrate and glucose concentrations in the culture medium were measured over time to observe the effect of citrate on glucose consumption. ATPase activity was measured to verify that energy production was regulated by CitAB. Furthermore, the growth-stimulating effect of citrate was verified using strains deficient in genes associated with citrate metabolism and anaerobic respiration. In summary, we determined the mechanism of anaerobic respiration using citrate and CitAB.

Results

The gSELEX method successfully identified approximately 30 CitB-binding regions in the *E. coli* genome (Fig. 1). All previously reported CitB target genes were identified. Several novel target genes involved in carbon metabolism and respiration were also identified. These genes were selected as the targets for this study. The ability of CitB to bind to these novel targets was verified using a gel shift assay, and it was confirmed that CitB specifically bound to all targets.

To further examine the effect of CitAB on transcriptional regulation, the expression levels of the novel target genes were compared in wild-type and *citAB*-deficient *E. coli* strains in glucose minimal medium under anaerobic conditions, with or without the addition of citrate. It was found that the mRNA levels of all target genes increased more than 2-fold in the wild-type strain upon the addition of citrate.



CitB Intensity	center	RegulonDB	Left Function	Left operon	LeftGene		CitB		RightGene	Right operon	Right Function
79.6	651344	Known	citrate lyase synthetase	*citCDEFXG*	*citC*	<		>	*citA*	*citAB*	DpiB sensory histidine kinase
55.3	29360	Newly	DapB	*dapB*	*dapB*	>		>	*carA*	*carAB*	CarA
32.2	121872	Newly	phenylalanine/tyrosine/tryptophan APC transporter	*aroP*	*aroP*	<		>	*pdhR*	*pdhR-aceEF-lpd*	PdhR transcriptional dual regulator
28.6	953940	Newly	FocA formate FNT transporter	*focA-pflB*	*focA*	<		<	*ycaO*	*ycaO*	conserved protein
26.4	3921034	Newly	AtpI	*atpIBEFHAGDC*	*atpI*	<		<	*rsmG*	*asnC-mioC-mnmG-rsmG*	16S rRNA m7G527 methyltransferase
14.5	4347168	Newly	DcuB dicarboxylate Dcu transporter	*dcuB-fumB*	*dcuB*	<		<	*dcuR*	*dcuSR*	DcuR transcriptional activator
12.4	3382448	Known	Mdh	*mdh*	*mdh*	<		>	*argR*	*argR*	ArgR

Figure 1. CitB-binding regions on the *E. coli* genome, and representative target genes and their functions. The horizontal axis shows the position of the *E. coli* genome and the vertical axis shows the CitB binding intensities. Green indicates known targets and white indicates newly targets.

Cell growth was monitored under the same conditions as above. The addition of citrate enhanced the growth of the wild-type strain, but no effect was observed in the *citAB*-deficient strain. When the concentrations of citrate, succinate, and glucose in the medium under these conditions were measured over time, accelerated succinate production and glucose consumption were observed with the consumption of citrate in the wild-type strain, but not in the *citAB*-deficient strain. These results demonstrated a citrate- and *citAB*-dependent increase in the growth rate by promoting glucose consumption. Furthermore, as the transcription of genes encoding ATP synthase was activated by CitAB, ATP synthase activity was measured under the same conditions. The results indicated that ATP synthase activity is dependent on both citrate and CitAB. ATP production by ATP synthases requires a proton concentration gradient. Therefore, the growth-promoting effect of citrate was observed in strains deficient in respiration-related genes and was abolished in strains deficient in fumarate reductase and NADH:quinone oxidoreductase.

Conclusion

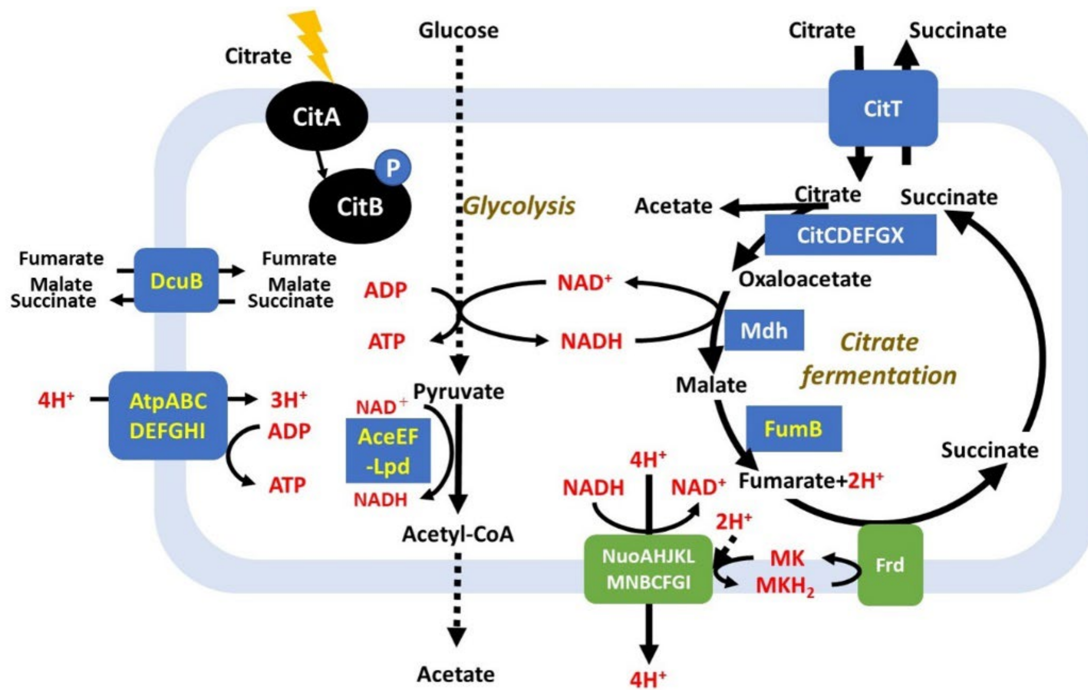


Figure 2. Model of the novel regulation of energy production by citrate anaerobic respiration by citrate-responsive two-component system CitAB in *E. coli*

The results of this study suggest that the role of the citrate-responsive two-component system CitAB regulatory network in *E. coli* is not only the consumption of reducing power (NADH) by “citrate fermentation” under anaerobic conditions, but also the activation of glycolysis by activating pyruvate dehydrogenase (AceEF-Lpd) and ATP synthase (AtpABCDEFGHI) to produce ATP using a proton concentration gradient (Fig. 2).

References

1) Shimada, T., Ogasawara, H. and Ishihama, A. (2018) Genomic SELEX screening of regulatory targets of *Escherichia coli* transcription factors. *Methods Mol. Biol.* **1837**: 49-69.