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Metabolic control of the TCA cycle by the YdcI transcriptional regulator in *Escherichia coli*

Yousuke Nishio

Institute for Innovation

AJINOMOTO Co., Inc.

Agenda

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- Background
  - Why is TCA cycle important?
- Bioinformatics approach for network finding
  - DNA motif finding process
- Functional validation of YdcI in *E. coli*
  - L-Glu fermentation

Key messages

- Network analysis will provide attractive hypothesis which is worthy of the experimental validation.
- Basic idea in fermentation study will provide metabolism based understanding in bacteriology.

Background : fermentation technology

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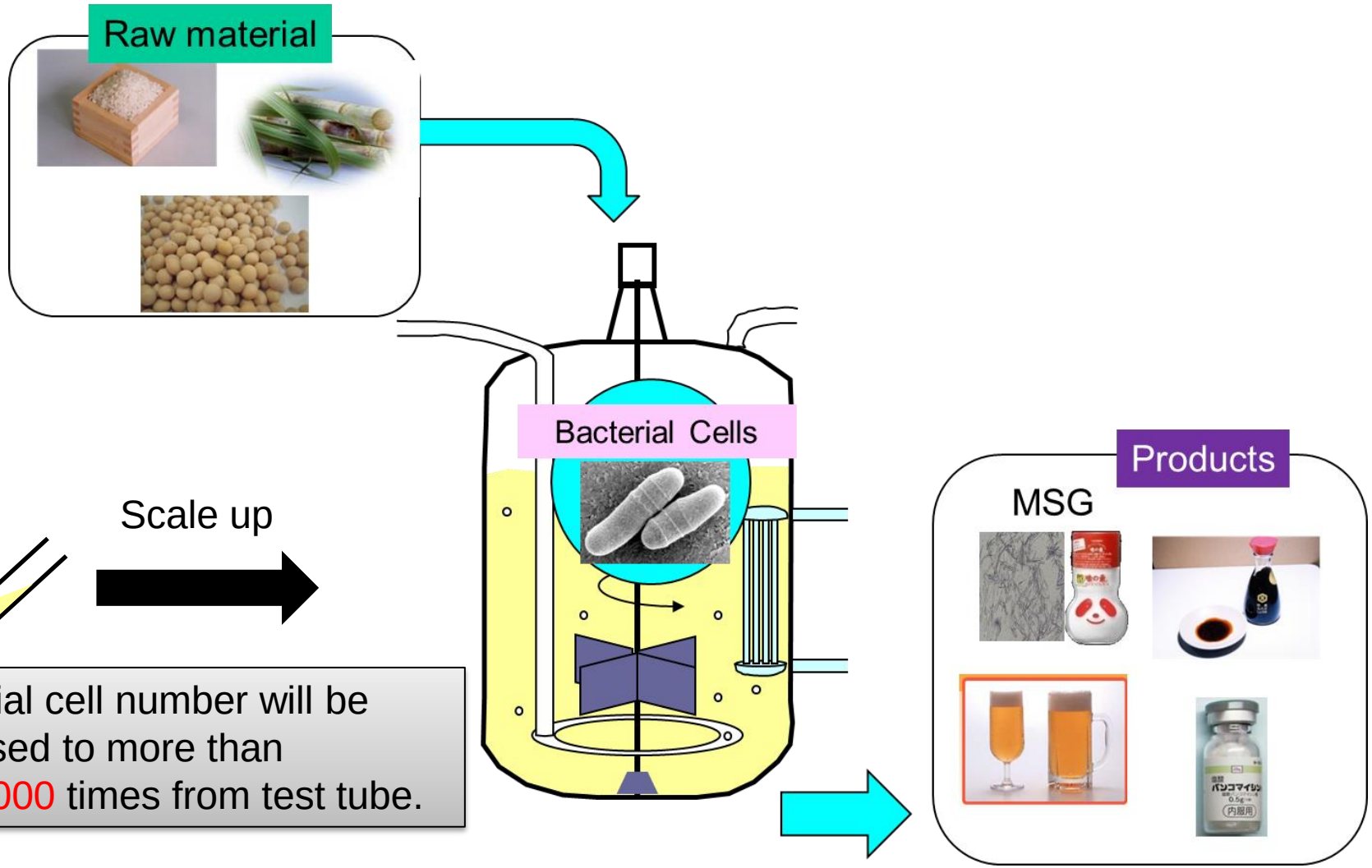

Bacterial cell number will be increased to more than 7,500,000 times from test tube.

By controlling bacterial cell growth and material production, we have developed industrial fermentation technology.

Formulation of metabolism in fermentation

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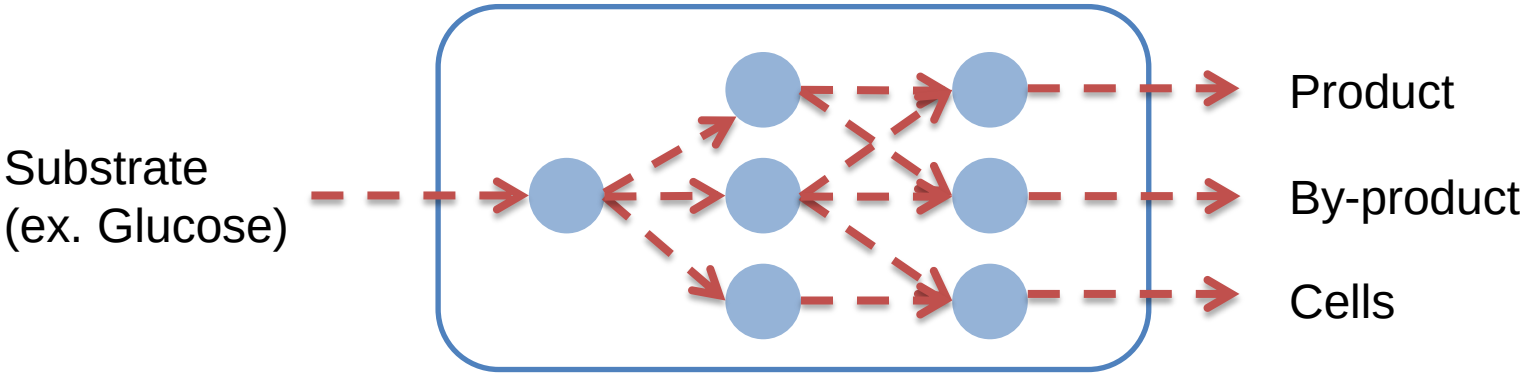
General concept of metabolism in fermentation

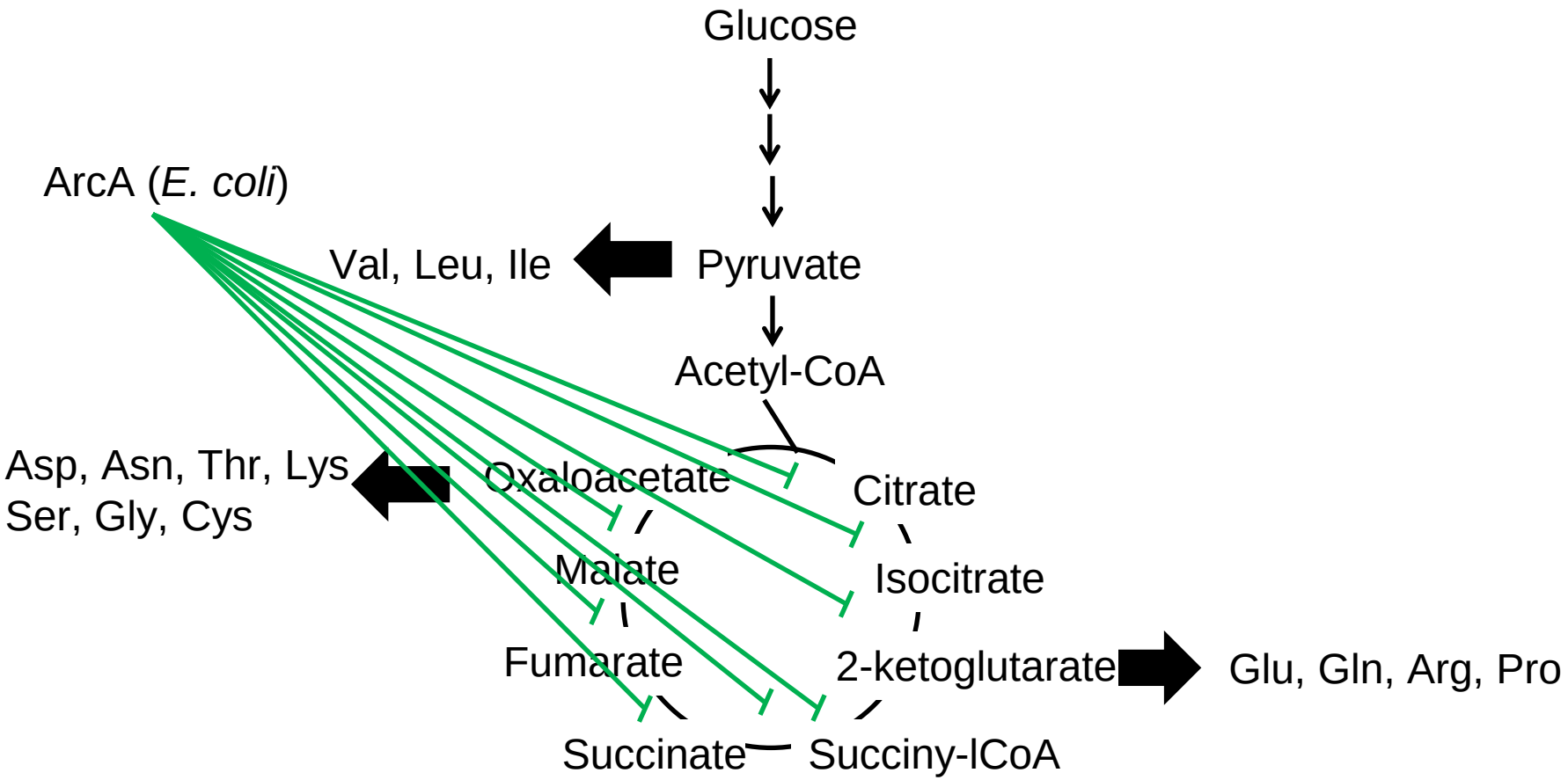


Stoichiometry for cells in aerobic fermentation condition

$$Biomass\ formation = \sum precursor + \sum energy + \sum oxygen$$

- Protein, DNA, RNA, lipid, murein and glycogen are necessary for biomass formation. These molecules are provided through metabolic reactions.
- Energy molecule (ATP, NAD(P)H) are provided through metabolic reactions and respiration.

Amino acid fermentation by *E. coli*



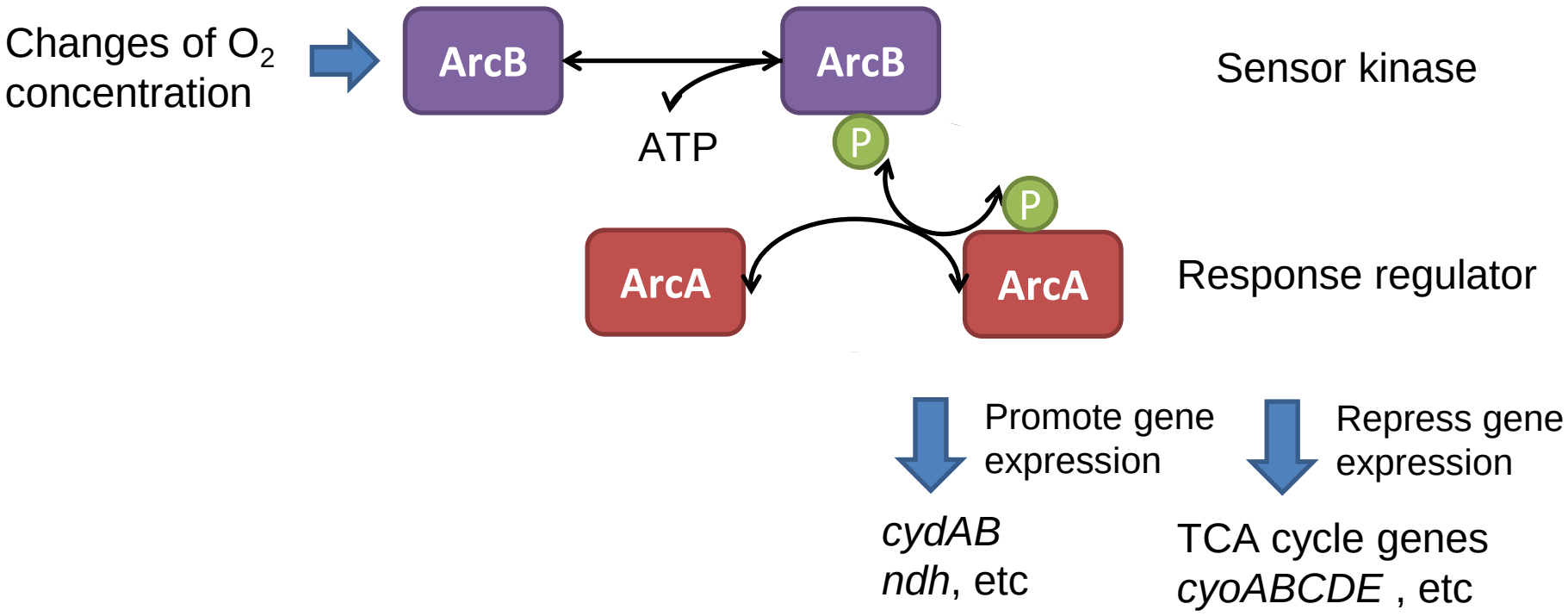
Stoichiometry for complete oxidation of pyruvate in TCA cycle

$$Pyruvate + 3\ H_2O + GDP + 2{\sim}P + 4\ NAD^+ + FAD = 3\ CO_2 + GTP + 4\ NADH + FADH + 4\ H^+$$

Combine with respiration, complete oxidation of pyruvate generates 15 ATP molecules.

Regulation of carbon flux into TCA cycle is important for cell growth control and amino acid fermentation.

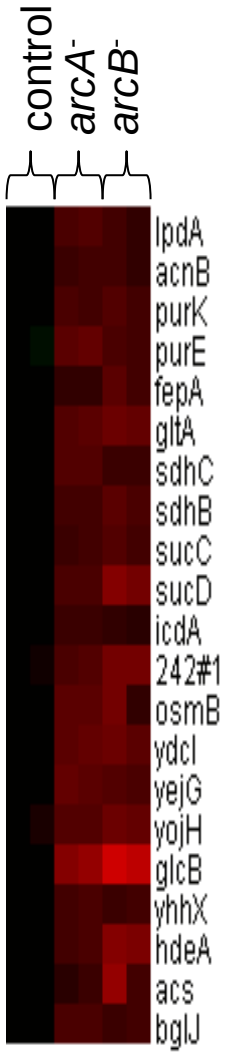
ArcAB two-component system in *E. coli*



- Gene disruption of *arcA* has been shown to enhance expression of TCA cycle genes. As a result, carbon flux into TCA cycle will be increased.
- Gene amplification of *arcA* may not repress expression of TCA cycle genes.

Main purpose of this study
Finding out regulatory factor which represses carbon flux into TCA cycle

DNA array data analysis



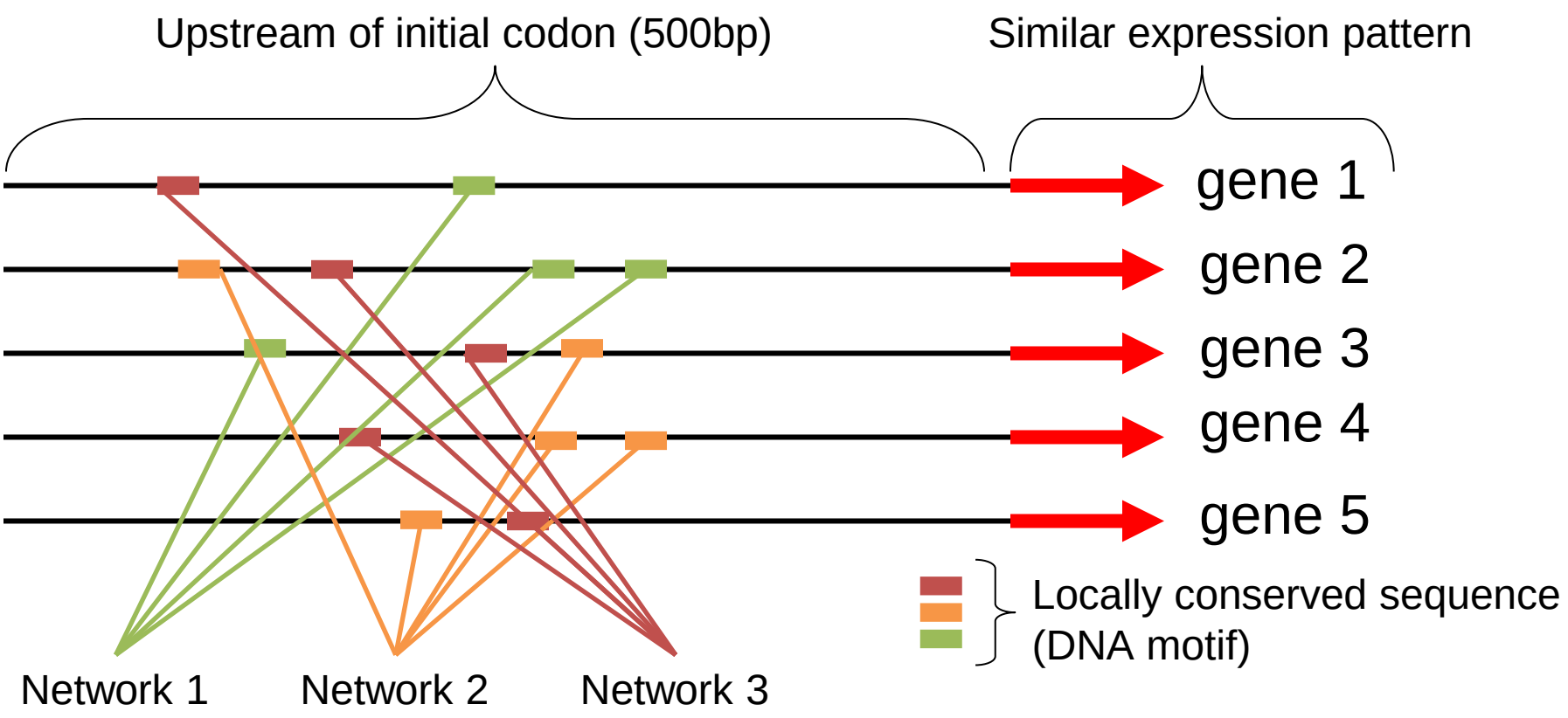
【DNA array data analysis result】
By data analysis of Δ*arcA* and Δ*arcB* gene in *E. coli*[1)], we extracted genes whose expression are negatively regulated by ArcA.

【Result】
Among 21 genes whose expression levels were increased, only 9 genes (*lpdA*, *acnB, gltA*, *sdhCB,sucCD*, *icdA,* and *glcB*) have been known as ArcA regulon. We observed the increase of expression levels due to unknown regulation system.

【Working hypothesis】
Through gene network analysis of these 21 genes, we may find out new regulation system involved in TCA cycle regulation.

1) Oshima, T. et al., *Mol Microbiol*, **46**, 281-291, 2002

Gene network analysis



- Key concept is finding out all of DNA motifs
- Network formation is defined per DNA motif

DNA motif detection

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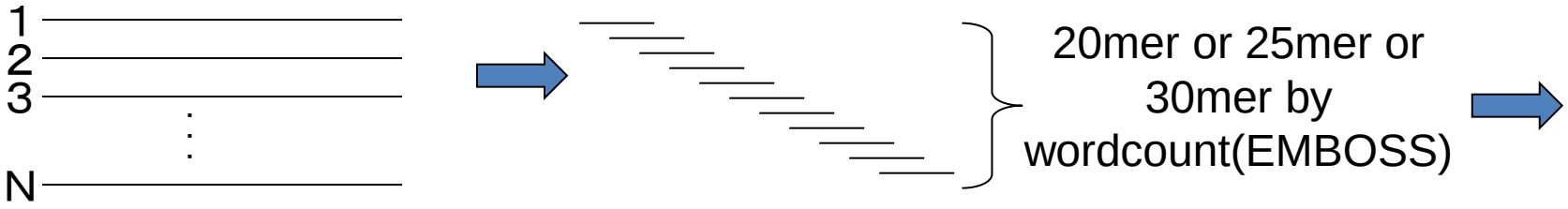
Step 1 : input sequence

Step 2 : breakdown to elements

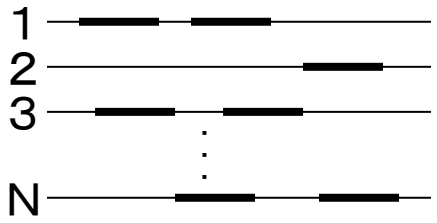


Step 3 : finding similar sequence

Step 4 : aligning elements

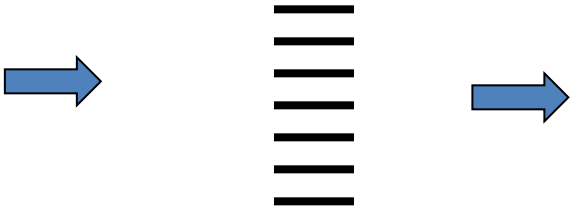
Step 5 : weak similarity

Input sequence

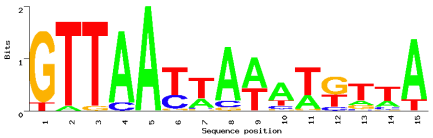


20mer, 25mer or 30mer elements will have similar sequence in input sequence.
fuzznuc (EMBOSS)

DNA motif



Multiple Alignment by MEME



Finding similar sequence by HMMalign (MATLAB)

Belonging to the same gene network

DNA motif comparison

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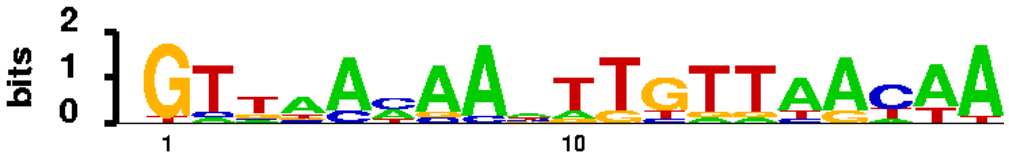
Motif A



Motif B

Information content matrix for Motif A

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A	0	−0.133	−0.133	0.8578	1.3995	0.1966	1.3995	1.6932	0.392	−0.084	0	0	−0.133	−0.133	1.1204	1.3995	−0.133	1.1204	1.6932
G	1.6932	0	−0.084	−0.133	0	0	−0.133	0	−0.133	−0.133	−0.084	1.1204	−0.133	−0.133	0	−0.084	0	0	0
T	−0.133	1.3995	0.8578	−0.084	0	−0.084	0	0	0.0342	1.1204	1.3995	−0.084	1.3995	1.3995	−0.084	0	0.0342	0.0342	−0.133
C	0	−0.133	−0.133	−0.133	−0.084	0.392	−0.133	−0.133	−0.084	0	0	−0.133	0	0	−0.133	0	0.8578	0	0

Information content matrix for Motif B

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	0	−0.118	0	1.6459	2	0	0.0195	1.4754	0.7014	0.8444	0.1064	0	−0.105	0.0195	1.4754
G	1.6459	0	−0.118	0	0	−0.118	−0.118	−0.118	0	−0.118	−0.118	0.5653	−0.105	−0.118	0
T	−0.131	1.8208	1.8208	0	0	0.8444	0.8444	0	0.3167	−0.052	0.8444	0.2061	0.8444	0.7014	−0.105
C	0	0	0	−0.131	0	0.1064	−0.118	−0.131	0	−0.131	0	−0.131	−0.118	−0.131	0

[Information content calculation]

$$\mathrm{IC} = \sum_{b=A}^{T} f_b \log_2 \frac{f_b}{p_b}$$

f_b is the appearance frequency of A, G, C and T in a residue position of the DNA motif, and P_b is the A, G, C, and T frequencies in the *E. coli* genome, which were each set to 25%.

DNA motif comparison

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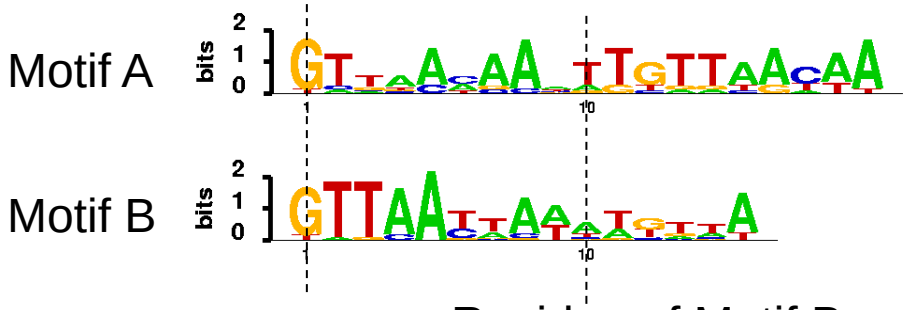


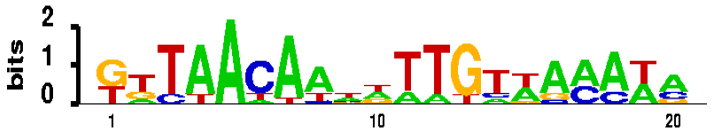
Similarity score = 19.58

Residue of Motif B

Residue of Motif A	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0	−2	−4	−6	−8	−10	−12	−14	−16	−18	−20	−22	−24	−26	−28	−30
1	0	2.804	0.804	−1.2	−3.2	−5.2	−7.2	−9.2	−11.2	−13.2	−15.2	−17.2	−19.2	−21.2	−23.2	−25.2
2	0	0.804	5.368	3.368	1.368	−0.63	−2.63	−4.63	−6.63	−8.63	−10.6	−12.6	−14.6	−16.6	−18.6	−20.6
3	0	−0.25	3.368	6.94	4.94	2.94	0.94	−1.06	−3.06	−5.06	−7.06	−9.06	−11.1	−13.1	−15.1	−17.1
4	0	−0.21	1.368	4.94	8.369	6.656	4.656	2.656	0.655	−1.34	−3.34	−5.34	−7.34	−9.34	−11.3	−13.3
5	0	0	−0.37	2.94	7.254	11.17	9.168	7.168	5.168	3.168	1.168	−0.83	−2.83	−4.83	−6.83	−8.83
6	0	0.011	−0.18	0.94	5.254	9.168	11.14	9.139	7.407	5.407	3.407	1.407	−0.59	−2.59	−4.59	−6.53
7	0	−0.22	−0.15	−0.16	3.261	8.053	9.17	11.2	11.24	9.237	7.237	5.237	3.237	1.237	−0.76	−2.53
8	0	0	−0.42	−0.15	2.645	6.647	8.039	9.218	13.71	12.43	10.69	8.685	6.685	4.685	2.685	1.736
9	0	−0.22	0.016	−0.34	0.645	4.647	6.683	8.101	11.71	14	12.78	10.78	8.781	6.781	4.781	3.259
10	0	−0.37	1.827	2.072	0.072	2.647	5.609	7.643	9.714	12.01	13.89	13.73	11.73	9.75	7.75	5.75
11	0	−0.32	2.183	4.385	2.385	0.647	3.839	6.801	7.714	10.16	11.95	15.08	13.98	12.92	10.92	8.924
12	0	1.855	0.183	2.385	4.403	2.403	1.839	4.801	6.686	8.157	10.05	13.08	15.71	13.8	12.75	10.93
13	0	−0.14	4.419	2.747	2.403	4.137	3.6	3.034	4.686	7.036	8.046	11.23	13.71	16.92	14.92	12.92
14	0	−0.4	2.419	6.983	4.983	2.983	5.335	4.795	2.854	5.036	6.866	9.229	11.71	14.92	17.92	15.92
15	0	0.011	0.419	4.983	8.845	7.224	5.224	5.302	6.466	4.466	6.004	7.229	9.711	12.92	15.92	19.58
16	0	−0.14	−0.15	2.983	7.286	11.64	9.644	7.644	7.377	7.447	5.657	6.162	7.711	10.92	13.92	17.98
17	0	−0	−0.06	0.983	5.286	9.644	11.76	9.764	7.764	7.294	7.221	5.672	6.057	8.921	11.92	15.98
18	0	−0	−0.07	0.003	3.286	7.644	9.764	11.81	11.42	9.417	8.239	7.369	5.679	6.921	9.916	13.98
19	0	0.017	−0.45	−0.32	2.79	6.673	7.764	9.814	14.31	12.56	10.85	8.854	7.342	5.39	7.916	12.43

DNA motifs were classified into two groups based on their similarities.

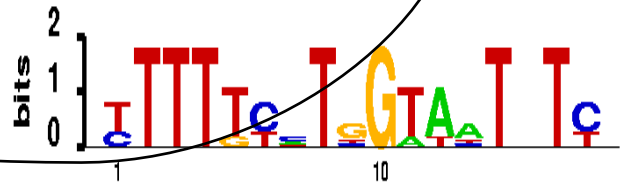
Predicted gene network



lpd *acnB* *fepA* *gltA* *sdhCB,sucCD* *ydcI* *yejG* *mqo* *b1163* *osmB* *glcB* *acs*

New knowledge

YdcI



Networking by DNA motif

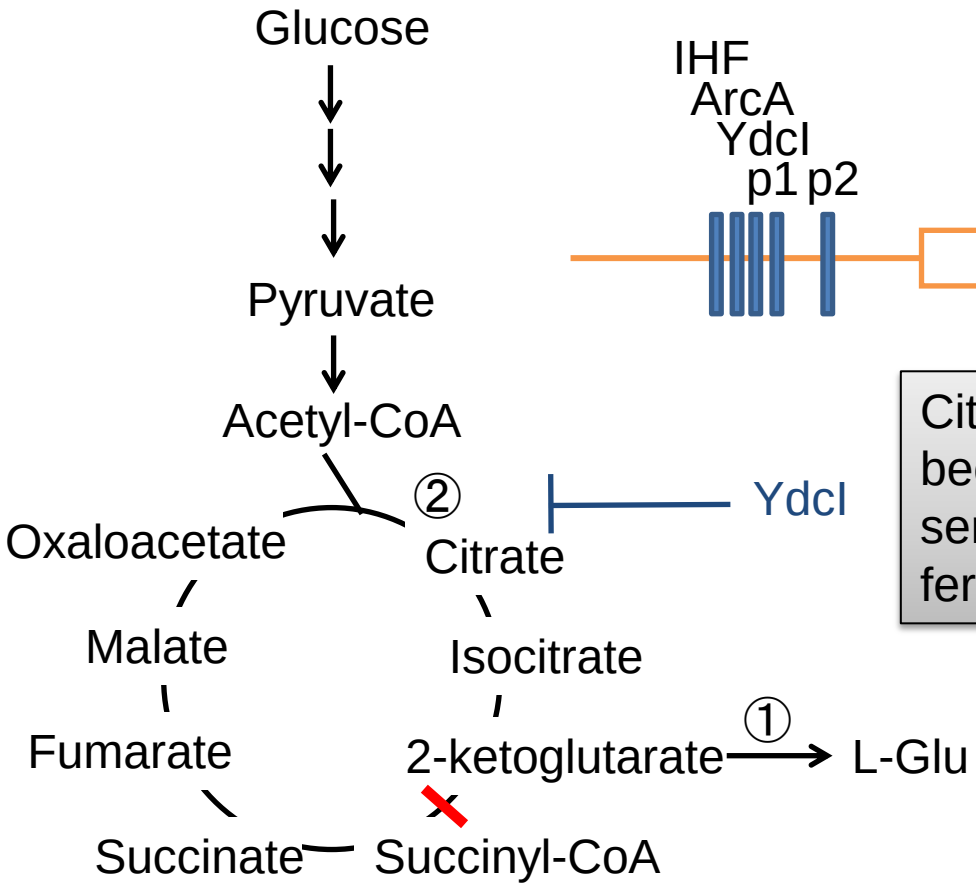
Weak similarity

icdA *yhhX* *hdeA* *purEK* *bglJ*

Disagreement with previous knowledge

- YdcI is a LysR family predicted HTH transcriptional regulator
- YdcI is suggested to regulate gene expression on TCA cycle.
- Attractive hypothesis is proposed by DNA motif based gene network.

Model for experimental validation

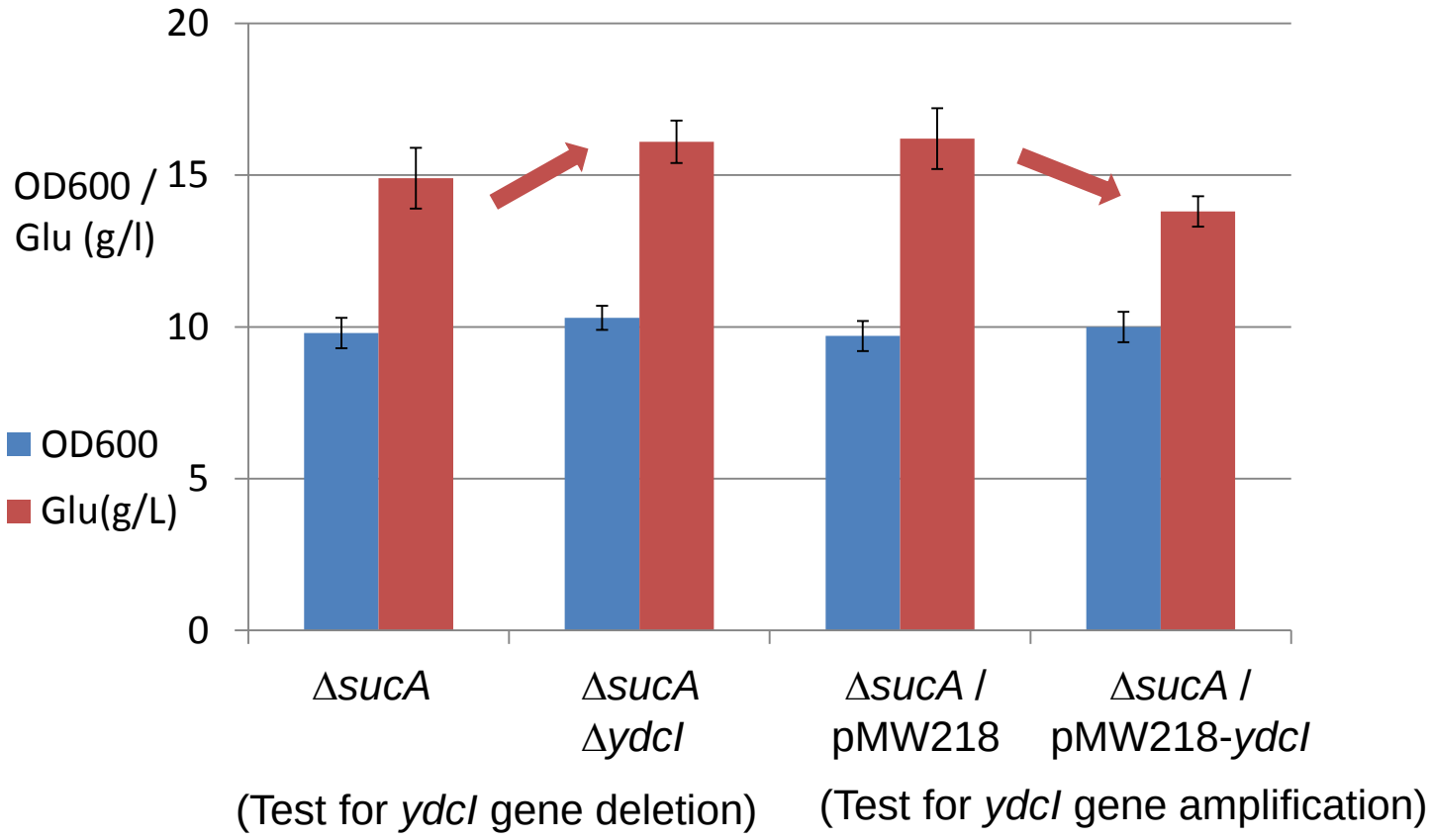


Citrate synthase activity has been known to show high sensitivity for L-Glu fermentation in *E. coli* [(1)].

①Confirm the effect of *ydcI* gene amplification or deletion on L-Glu fermentation
②Confirm the effect of *ydcI* gene amplification or deletion on citrate synthase activity

(1) Nishio Y et al., Analysis of l-glutamic acid fermentation by using a dynamic metabolic simulation model of *Escherichia coli*, BMC Systems Biology 2013, **7**:92

L-Glu fermentation result



- Condition: glucose 40g/L, MS medium, 24h & 37°C cultivation
- Cell growth (OD600) should not be different in this condition because of Δ*sucA* genotype.

L-Glu accumulation is increased by *ydcI* gene deletion & decreased by *ydcI* gene amplification

→ YdcI may control carbon flux into TCA cycle.

Relative activity of citrate synthase

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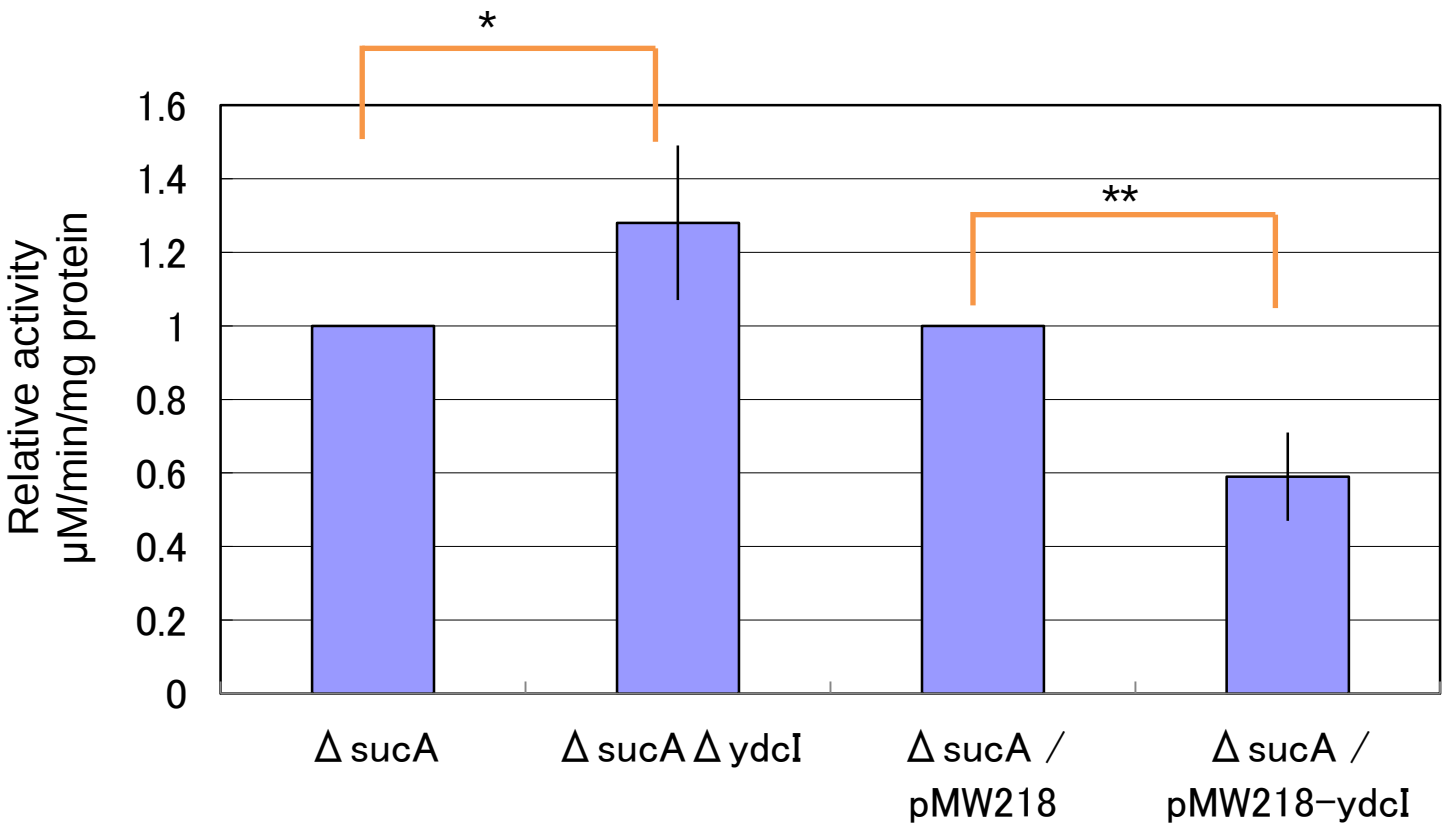
Oxaloacetate + Acetyl-CoA → Citrate + CoA
CoA + DTNB → TNB + CoA-derivative
Increase of 412nm absorbance

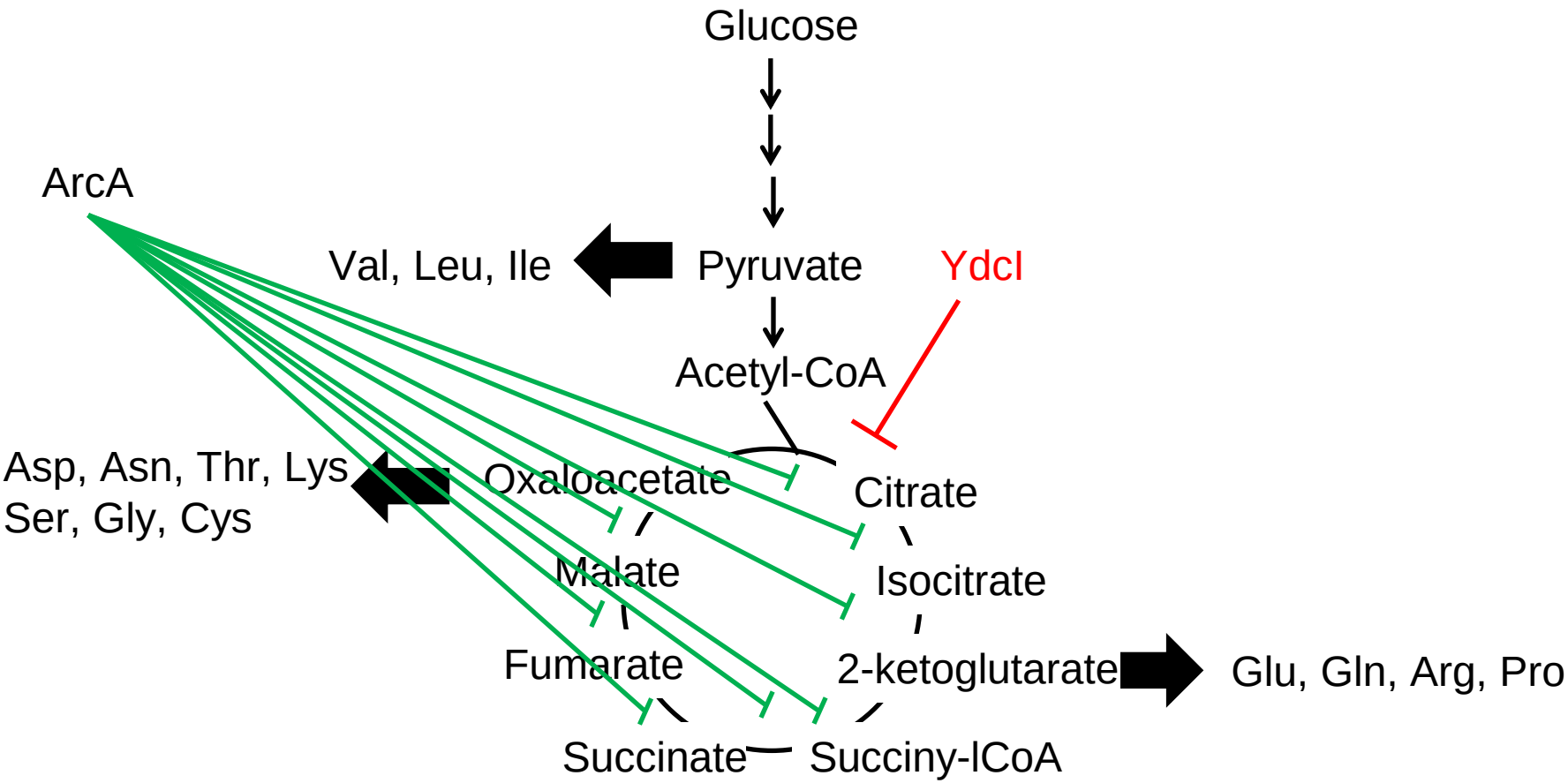


* : $p<0.05$
** : $p<0.01$

Expression level of citrate synthase is increased by *ydcI* gene deletion and decreased by *ydcI* gene amplification

YdcI may control citrate synthase expression levels by regulating *gltA* gene expression.

Proposed TCA cycle regulation in *E. coli*



- Protein-DNA interaction at promoter region of *gltA* gene should be verified.
- Cell growth control by YdcI should be verified.

Summary

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- Combination of DNA array and DNA motif analysis is useful for understanding gene network.
  - Similarity scores between DNA motifs were calculated using the information content of the DNA motif and semi-global alignment with no penalty for end gaps.
- Biological function of YdcI in *E. coli* was proposed as a transcriptional regulator repressing *gltA* gene.

Nishio Y, Suzuki T, Matsui K, Usuda Y (2013) Metabolic Control of the TCA cycle by the YdcI Transcriptional Regulator in *Escherichia coli*.

J Microb Biochem Technol 5: 059-067. doi:10.4172/1948-5948.1000101

Acknowldgement

- Dr. Yoshihiro Usuda
- Mr. Yohei Yamada
- Ms. Tomoko Suzuki

YdcI binding test on *gltA* promoter region

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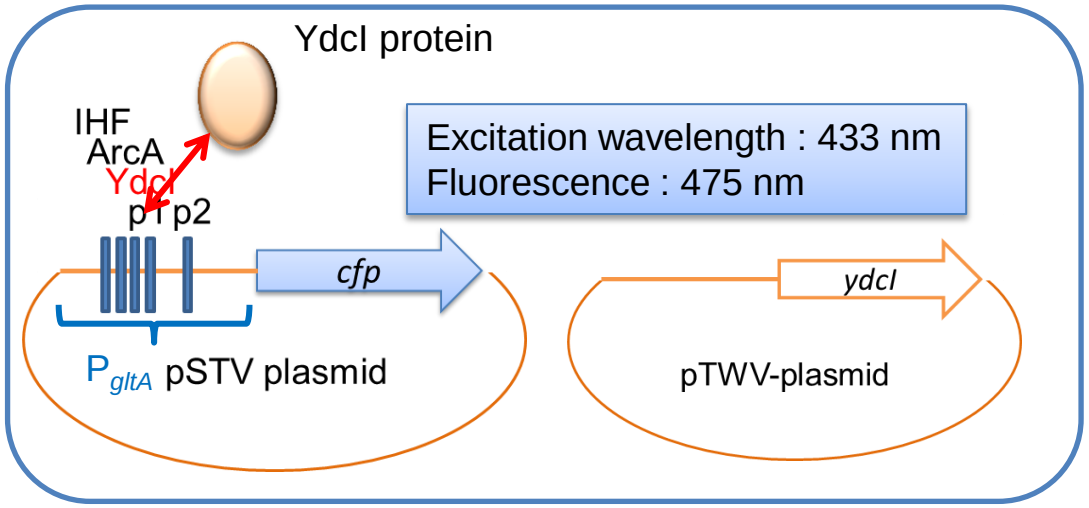
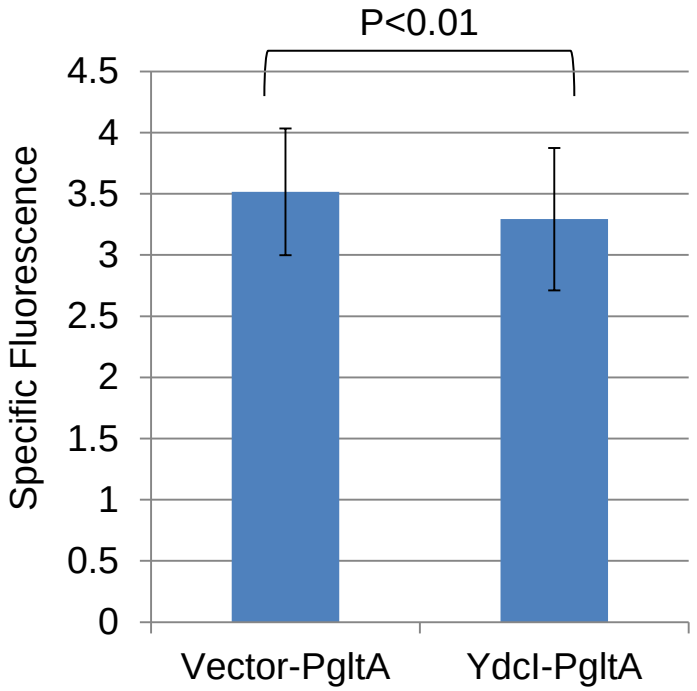
[Experimental condition]



- *E. coli* BW25113 was used as a host.



- There was statistically significant difference in specific fluorescence, although observed difference was not large.
- YdcI may bind to promoter region of *gltA* gene.

Discussion (1) YdcI-DNA interaction

- Dr. Andrea Veit found YdcI-Psdh interaction in her PhD thesis.

Another regulator of the *sdhCDAB*-b0725-*sucABCD*-operon in *E. coli* MG1655 could be identified by DNA-affinity chromatography: YdcI. This regulator which belongs to the lysR family was isolated from cells grown in glucose minimal medium. YdcI is supposed to take part in the regulation of aerobic acetate formation.

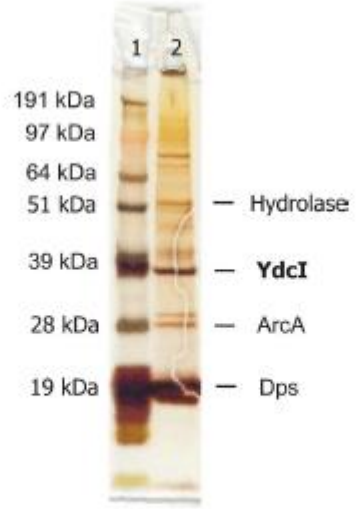


Abb. 25: Silbergefärbtes SDS - Gel der Proteine, die durch DNA - Affinitätschromatographie aus einem Proteinextrakt von *E. coli* MG1655 angereichert wurden. Spur 1: Proteinstandard See BlueII; Spur 2: Proteinprobe.

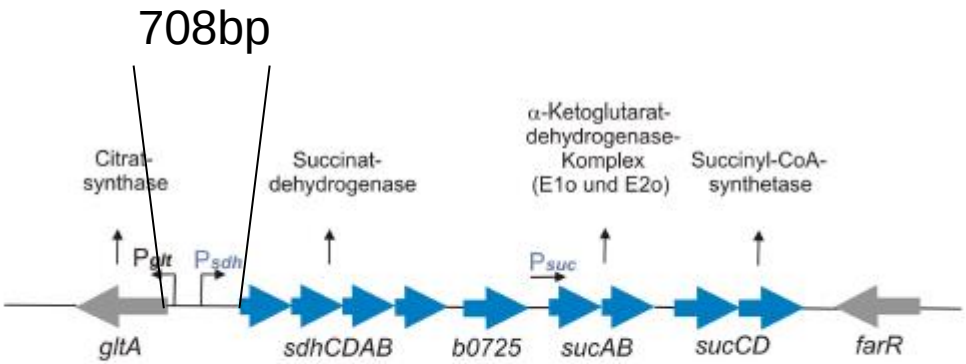


Abb. 6: *sdhCDAB-b0725-sucABCD* - Operon in *Escherichia coli* (P*glt* =Citratsynthase-Promotor, P*sdh* = *sdh* -Promoter, P*suc* =*suc* -Promoter, E1o und E2o=Decarboxylase- und Transacylase-Komponenten des α-Ketoglutaratdehydrogenase-Enzymkomplexes, siehe auch Text).

Andrea Veit, Untersuchungen zum Überflussmetabolismus in *Escherichia coli, Band 4199 von Berichte des Forschungszentrums Jülich, Forschungszentrum Jülich, ISSN 0944-2952 Forschungszentrum, Zentralbibliothek, 2006*

Discussion (2) Cell growth control by YdcI

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Dr. J. W. Wilson has shown the effect of YdcI overexpression to cell growth in *E. coli*.

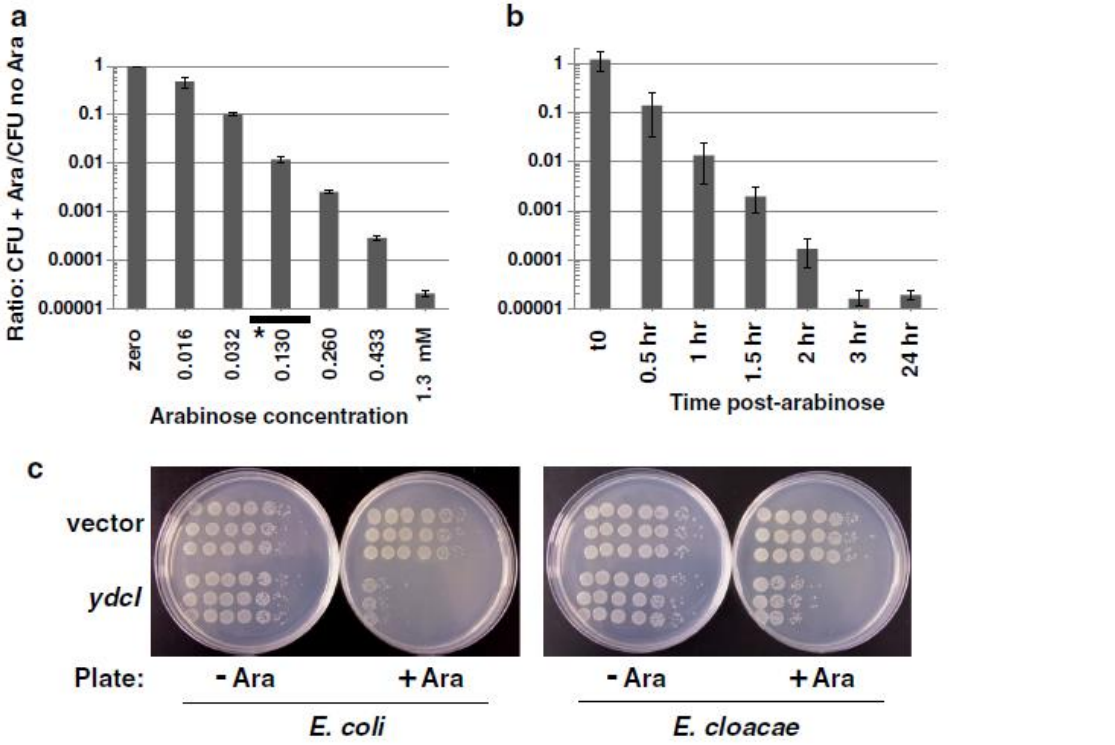

Fig. 3 Characterization of YdcI effects in non-tolerant bacteria. **a** Cultures of *E. coli* (pBAD + *ydcI*) were grown as in Figs. 1 and 2 in the presence of different arabinose concentrations, serial-diluted, and plated on LB agar media (containing no arabinose). The ratios of CFU in each arabinose culture to CFU in the zero arabinose culture were calculated and plotted as indicated. The solid line under the *X*-axis indicates the range of arabinose that overlaps with the physiological level of YdcI expression in *S.* Typhimurium as assayed via regulation of chromosomal *ydcI::lacZ* fusions shown in Fig. 5a. The *asterisk* under the *X*-axis indicates the level of arabinose (100 μM) corresponding to physiological *S.* Typhimurium YdcI levels as assayed via Western blot in Fig. 5b. **b** Cultures of *E. coli* (pBAD + *ydcI*) were grown as in Figs. 1 and 2 but plated at different times after addition of arabinose. The ratios of CFU in the plus arabinose culture to CFU in the zero arabinose culture at each time point were calculated and plotted as indicated. Arabinose was used at a concentration of 1.3 mM. **c** Broth cultures of *E. coli* or *E. cloacae* containing pBAD18 or pBAD18 + *ydcI* (vector and *ydcI*, respectively) were grown in the absence of arabinose, serially diluted, and plated on LB agar media with or without arabinose

L. Solomon *et al.*, Bacterial Genus-Specific Tolerance for YdcI Expression, *Curr. Microbiol*. **69**, 640-648, 2014

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