nature portfolio

Corresponding author(s): Anna Zawilak-Pawlik

Last updated by author(s): Sep 27, 2023

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	ImageQuant (v. 8.1), ImageLab (v. 5.2), Axiovision (v. 4.8).
Data analysis	Bowtie2 (v. 2.3.5.1), samtools (v. 1.10), Rsubread (v. 2.10), edgeR (v. 3.38), MACS3 (v.3.0) csaw (v. 1.30), MaxQuant (v. 1.6.3.4), CFX Maestro (v. 1.1), GraphPad Prism (v. 8.4.2), R (v. 4.2).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The RNA-seq FASTQ and processed data generated in this study have been deposited in the ArrayExpress database (EMBL-EBI) under accession code E-MTAB-13025 (https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-13025?key=bf2d8677-1b26-4f1e-ac5f-b1c78c7590be).
The ChIP-seq FASTQ and BED files generated in this study have been deposited in the ArrayExpress database (EMBL-EBI) under accession code E-MTAB-13026 (https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-13026?key=2b27b413-e118-4802-859e-5da57ff1ead2).
The raw proteomics data, MaxQuant search results, and the used protein sequence database generated in this study have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository under accession code PXD041978 (http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD041978).
Helicobacter pylori 26695 reference genome is deposited in the National Center for Biotechnology Infromation under accession code NC_000915.1 (www.ncbi.nlm.nih.gov/nuccore/NC_000915.1?report=genbank).
Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed. RNA-seq and ChIP-seq experiments were performed with 3 biologigal replicates base on the time, cost and convenience of data collection with sufficient statistical power. The proteomics experiment was performed with four biological replicates based on the time, cost and convenience of data collection with adequate statistical power.
Data exclusions	No data were excluded from the analysis.
Replication	Biological replicates were cultures started independently from a glycerol stock on different days. The RNA-seq and ChIP-seq data were collected from 3 independent experiments. The LC-MS/MS (proteomics) data were collected from 4 independent experiments. The microscopy (Cy3-λ DNA uptake) data were collected from at least 3 independent experiments. The transformation rate glucose uptake and relative ATP production data were collected from 3 independent experiments. EMSA and catalase assay were repeated two times. Only successful experiments were regarded as replicates.
Randomization	Randomization was not relevant to this investigation. All samples were subjected to the same screening methods.
Blinding	Blinding was not relevant to this investigation. All samples were subjected to the same screening methods.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Anti-HP1021 antibody: the immunoglobulin G (IgG) fraction of rabbit antibodies containing the anti-6HisHP1021 antibody was obtained by ammonium sulfate precipitation from rabbit serum. The antibody was prepared in our laboratory, approved by the First Local Committee for Experiments with the Use of Laboratory Animals, Wroclaw, Poland (permission number 51/2012).
Anti-rabbit antibody: rabbit IgG HRP-linked whole Ab, NA934 GE Healthcare, LOT 9494122.

Validation

The anti-HP1021 antibody was validated using extracts from bacterial strains mutated in the HP1021 gene (doi.org/10.1111/mmi.12866; Fig. 2 A).

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.

The ChIP-seq FASTQ and BED files have been deposited in the ArrayExpress database (EMBL-EBI) and can be accessed using the dataset identifier E-MTAB-13026 (https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-13026?key=2b27b413-e118-4802-859e- 5da57ff1ead2).

Files in database submission

D_1_1.fq.gz
D_1_2.fq.gz
D_2_1.fq.gz
D_2_2.fq.gz
D_3_1.fq.gz
D_3_2.fq.gz
D_4_1.fq.gz
D_4_2.fq.gz
D_5_1.fq.gz
D_5_2.fq.gz
D_6_1.fq.gz
D_6_2.fq.gz
R10_D_1.fq.gz
R10_D_2.fq.gz
R10S_D_1.fq.gz
R10S_D_2.fq.gz
R11_D_1.fq.gz
R11_D_2.fq.gz
R11S_D_1.fq.gz
R11S_D_2.fq.gz

R12_D_1.fq.gz
R12_D_2.fq.gz
R12S_D_1.fq.gz
R12S_D_2.fq.gz
peaks_ChIPseq_N6.bed

Genome browser session
(e.g. UCSC)

n/a

Methodology

Replicates

The ChIP-seq data were collected from 3 independent experiments (biological replications).

Sequencing depth

total number / uniquely mapped reads

D1 10163703/9332312
D2 11928945/10910213
D3 13599676/12440983
D4 13713527/12351773
D5 13542187/12418185
D6 11436611/10493090
R10 11079644/11079644
R10S 10953659/10179235
R11 17236594/15700813
R11S 14374049/13442610
R12 11173477/10248313
R12S 11522928/10490473

read lenght 150 bp, paired-end

Antibodies

anti-HP1021 antibody (for more details, see Antibodies section).

Peak calling parameters

Peak calling parameters:

bowtie2 - local
edgeR
width: 100 bp
spacing: 33 bp
filter type: localbin
length for filter: 2000 bp
minimal quality: 30
used glmQLFTest function with mergeWindows (merge distance = 100 bp, maximum peak length = 5000 bp), combineTests and getBestTest function

control files:
D1 D2 D3 to R10 R11 R12

Data quality

Read files were checked using fastqc.
ChIP-seq analysis followed established protocol. Peaks with logFC < 2 were removed from further analysis.
edgeR functions: combineTests and getBestTest were used to adjust for multiple comparisons.
Identified peaks were independently confirmed using MACS3 program.
edgeR: Peaks below FDR < 0.05 and logFC > 2 (all found peaks): WT vs ΔHP1021 56 (48) MACS3 WT vs ΔHP1021 82 (61).

Software

Data analysis reads were mapped to the H. pylori 26695 genome (NC_000915.1) using Bowtie2 (v. 2.3.5.1) and processed using samtools (v. 1.10). Regions differentially bound by HP1021 were identified using MACS3 (version 3.0.0a6) and R packages csaw (v. 1.30), and edgeR (v. 3.38).

2.1.1. Co-authors' declarations of publication I

10.07.2024

Declaration

I, Mateusz Noszka, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Kolenda, R., Meng, C., Ludwig, C., Stingl, K., Pawlik, A., *Profiling of the Helicobacter pylori redox switch HP1021 regulon using a multi-omics approach.* **Nature Communications** 14, 6715 (2023), doi.org/10.1038/s41467-023-42364-6,

is correctly characterized in the table below.

Contributor	Description of main tasks
Mateusz Noszka	**Conceptualization:** throughout project implementation. **Methodology:** implementing RNA-seq and ChIP-seq protocols for *Helicobacter pylori*. **Investigation:** conduction the experiments presented in this work, except EMSA assay, LC-MS/MS and NGS (RNA-seq and ChIP-seq) analyses and bioinformatics (both analyses performed on biological material I prepared); co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Formal analysis and Software:** statistical data analysis except for LC-MS/MS and NGS; NGS code modifications under Agnieszka Strzałka supervision. **Visualization**: preparation of all results for publication, including data collection, analysis (in cooperation with co-authors), and preparation of figures and tables - Fig. 1, 2, 3, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, Tab. S1, S2, Supplementary Data 1 and 2, Source data and Reporting summary. **Data curation:** Supplementary Data 1-2; RNA-seq and ChIP-seq data deposition in ArrayExpress. **Validation:** throughout project implementation. **Writing:** preparing a manuscript draft; reviewing and editing; preparing responses to reviewers. **Funding acquisition:** subproject 0000466 of the EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Agnieszka Strzałka	**Methodology, Formal analysis and Software:** bioinformatic NGS data analysis (RNA-seq and ChIP-seq). **Investigation and Visualization:** code preparation for most NGS and LC-MS/MS plots. **Supervision:** PhD student Mateusz Noszka in bioinformatic analyses. **Validation:** NGS data. **Writing:** original draft of NGS methodology; manuscript review and editing.
Jakub Muraszko	**Investigation:** EMSA assay; co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Writing:** manuscript review and editing.
Rafał Kolenda	**Formal analysis, Software, Methodology and Visualization:** code preparation for COG plots. **Writing:** manuscript review and editing.
Chen Meng	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Validation:** LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing.
Christina Ludwig	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing. **Validation:** LC-MS/MS data. **Data curation:** LC-MS/MS data deposition in ProteomeXchange Consortium. **Funding acquisition:** EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Kerstin Stingl	**Conceptualization, Methodology and Resources:** support in the DNA uptake assay. **Writing:** manuscript review and editing.
Anna Pawlik	**Conceptualization:** initial project proposal and throughout project implementation. **Visualization:** Supplementary Figure S12 and Supplementary

	Data 3. **Validation and Formal Analysis**: throughout project implementation. **Data curation:** Supplementary Data 3. **Supervision:** PhD student Mateusz Noszka. **Writing**: preparing a manuscript draft, reviewing and editing, and preparing responses to reviewers. **Funding acquisition and Project administration:** OPUS 17, Project Number 2019/33/B/NZ6/01648, founded by the National Science Centre, Poland.

(co-author signature)

10.07.2024

Declaration

I, Agnieszka Strzałka, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Kolenda, R., Meng, C., Ludwig, C., Stingl, K., Pawlik, A., *Profiling of the Helicobacter pylori redox switch HP1021 regulon using a multi-omics approach.* **Nature Communications** 14; 6715 (2023), doi.org/10.1038/s41467-023-42364-6,

is correctly characterized in the table below.

Contributor	Description of main tasks
Mateusz Noszka	**Conceptualization:** throughout project implementation. **Methodology:** implementing RNA-seq and ChIP-seq protocols for *Helicobacter pylori*. **Investigation:** conduction the experiments presented in this work, except EMSA assay, LC-MS/MS and NGS (RNA-seq and ChIP-seq) analyses and bioinformatics (both analyses performed on biological material I prepared); co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Formal analysis and Software:** statistical data analysis except for LC-MS/MS and NGS; NGS code modifications under Agnieszka Strzałka supervision. **Visualization**: preparation of all results for publication, including data collection, analysis (in cooperation with co-authors), and preparation of figures and tables - Fig. 1, 2, 3, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, Tab. S1, S2, Supplementary Data 1 and 2, Source data and Reporting summary. **Data curation:** Supplementary Data 1-2; RNA-seq and ChIP-seq data deposition in ArrayExpress. **Validation:** throughout project implementation. **Writing:** preparing a manuscript draft; reviewing and editing; preparing responses to reviewers. **Funding acquisition:** subproject 0000466 of the EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Agnieszka Strzałka	**Methodology, Formal analysis and Software:** bioinformatic NGS data analysis (RNA-seq and ChIP-seq). **Investigation and Visualization:** code preparation for most NGS and LC-MS/MS plots. **Supervision:** PhD student Mateusz Noszka in bioinformatic analyses. **Validation:** NGS data. **Writing:** original draft of NGS methodology; manuscript review and editing.
Jakub Muraszko	**Investigation:** EMSA assay; co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Writing:** manuscript review and editing.
Rafał Kolenda	**Formal analysis, Software, Methodology and Visualization:** code preparation for COG plots. **Writing:** manuscript review and editing.
Chen Meng	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Validation:** LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing.
Christina Ludwig	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing. **Validation:** LC-MS/MS data. **Data curation:** LC-MS/MS data deposition in ProteomeXchange Consortium. **Funding acquisition:** EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Kerstin Stingl	**Conceptualization, Methodology and Resources:** support in the DNA uptake assay. **Writing:** manuscript review and editing.
Anna Pawlik	**Conceptualization:** initial project proposal and throughout project implementation. **Visualization:** Supplementary Figure S12 and Supplementary

	Data 3. **Validation and Formal Analysis**: throughout project implementation. **Data curation:** Supplementary Data 3. **Supervision:** PhD student Mateusz Noszka. **Writing**: preparing a manuscript draft, reviewing and editing, and preparing responses to reviewers. **Funding acquisition and Project administration:** OPUS 17, Project Number 2019/33/B/NZ6/01648, founded by the National Science Centre, Poland.

A. Skrastha

.....

(co-author signature)

10.07.2024

Declaration

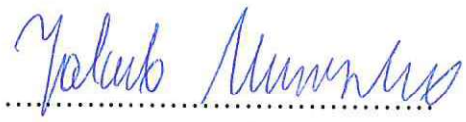
I, Jakub Muraszko hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Kolenda, R., Meng, C., Ludwig, C., Stingl, K., Pawlik, A., *Profiling of the Helicobacter pylori redox switch HP1021 regulon using a multi-omics approach.* **Nature Communications** 14, 6715 (2023), doi.org/10.1038/s41467-023-42364-6,

is correctly characterized in the table below.

Contributor	Description of main tasks
Mateusz Noszka	**Conceptualization:** throughout project implementation. **Methodology:** implementing RNA-seq and ChIP-seq protocols for *Helicobacter pylori*. **Investigation:** conduction the experiments presented in this work, except EMSA assay, LC-MS/MS and NGS (RNA-seq and ChIP-seq) analyses and bioinformatics (both analyses performed on biological material I prepared); co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Formal analysis and Software:** statistical data analysis except for LC-MS/MS and NGS; NGS code modifications under Agnieszka Strzałka supervision. **Visualization**: preparation of all results for publication, including data collection, analysis (in cooperation with co-authors), and preparation of figures and tables - Fig. 1, 2, 3, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, Tab. S1, S2, Supplementary Data 1 and 2, Source data and Reporting summary. **Data curation:** Supplementary Data 1-2; RNA-seq and ChIP-seq data deposition in ArrayExpress. **Validation:** throughout project implementation. **Writing:** preparing a manuscript draft; reviewing and editing; preparing responses to reviewers. **Funding acquisition:** subproject 0000466 of the EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Agnieszka Strzałka	**Methodology, Formal analysis and Software:** bioinformatic NGS data analysis (RNA-seq and ChIP-seq). **Investigation and Visualization:** code preparation for most NGS and LC-MS/MS plots. **Supervision:** PhD student Mateusz Noszka in bioinformatic analyses. **Validation:** NGS data. **Writing:** original draft of NGS methodology; manuscript review and editing.
Jakub Muraszko	**Investigation:** EMSA assay; co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Writing:** manuscript review and editing.
Rafał Kolenda	**Formal analysis, Software, Methodology and Visualization:** code preparation for COG plots. **Writing:** manuscript review and editing.
Chen Meng	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Validation:** LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing.
Christina Ludwig	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing. **Validation:** LC-MS/MS data. **Data curation:** LC-MS/MS data deposition in ProteomeXchange Consortium. **Funding acquisition:** EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Kerstin Stingl	**Conceptualization, Methodology and Resources:** support in the DNA uptake assay. **Writing:** manuscript review and editing.
Anna Pawlik	**Conceptualization:** initial project proposal and throughout project implementation. **Visualization:** Supplementary Figure S12 and Supplementary

	Data 3. **Validation and Formal Analysis**: throughout project implementation. **Data curation:** Supplementary Data 3. **Supervision:** PhD student Mateusz Noszka. **Writing**: preparing a manuscript draft, reviewing and editing, and preparing responses to reviewers. **Funding acquisition and Project administration:** OPUS 17, Project Number 2019/33/B/NZ6/01648, founded by the National Science Centre, Poland.



(co-author signature)

10.07.2024

Declaration

I, Rafał Kolenda, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Kolenda, R., Meng, C., Ludwig, C., Stingl, K., Pawlik, A., *Profiling of the Helicobacter pylori redox switch HP1021 regulon using a multi-omics approach.* **Nature Communications** 14, 6715 (2023), doi.org/10.1038/s41467-023-42364-6,

is correctly characterized in the table below.

Contributor	Description of main tasks
Mateusz Noszka	**Conceptualization:** throughout project implementation. **Methodology:** implementing RNA-seq and ChIP-seq protocols for *Helicobacter pylori*. **Investigation:** conduction the experiments presented in this work, except EMSA assay, LC-MS/MS and NGS (RNA-seq and ChIP-seq) analyses and bioinformatics (both analyses performed on biological material I prepared); co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Formal analysis and Software:** statistical data analysis except for LC-MS/MS and NGS; NGS code modifications under Agnieszka Strzałka supervision. **Visualization**: preparation of all results for publication, including data collection, analysis (in cooperation with co-authors), and preparation of figures and tables - Fig. 1, 2, 3, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, Tab. S1, S2, Supplementary Data 1 and 2, Source data and Reporting summary. **Data curation:** Supplementary Data 1-2; RNA-seq and ChIP-seq data deposition in ArrayExpress. **Validation:** throughout project implementation. **Writing:** preparing a manuscript draft; reviewing and editing; preparing responses to reviewers. **Funding acquisition:** subproject 0000466 of the EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Agnieszka Strzałka	**Methodology, Formal analysis and Software:** bioinformatic NGS data analysis (RNA-seq and ChIP-seq). **Investigation and Visualization:** code preparation for most NGS and LC-MS/MS plots. **Supervision:** PhD student Mateusz Noszka in bioinformatic analyses. **Validation:** NGS data. **Writing:** original draft of NGS methodology; manuscript review and editing.
Jakub Muraszko	**Investigation:** EMSA assay; co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Writing:** manuscript review and editing.
Rafał Kolenda	**Formal analysis, Software, Methodology and Visualization:** code preparation for COG plots. **Writing:** manuscript review and editing.
Chen Meng	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Validation:** LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing.
Christina Ludwig	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing. **Validation:** LC-MS/MS data. **Data curation:** LC-MS/MS data deposition in ProteomeXchange Consortium. **Funding acquisition:** EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Kerstin Stingl	**Conceptualization, Methodology and Resources:** support in the DNA uptake assay. **Writing:** manuscript review and editing.
Anna Pawlik	**Conceptualization:** initial project proposal and throughout project implementation. **Visualization:** Supplementary Figure S12 and Supplementary

	Data 3. **Validation and Formal Analysis**: throughout project implementation. **Data curation:** Supplementary Data 3. **Supervision:** PhD student Mateusz Noszka. **Writing**: preparing a manuscript draft, reviewing and editing, and preparing responses to reviewers. **Funding acquisition and Project administration:** OPUS 17, Project Number 2019/33/B/NZ6/01648, founded by the National Science Centre, Poland.

Rafał Kolenda

(co-author signature)

18.07.2024

Declaration

I, Chen Meng, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Kolenda, R., Meng, C., Ludwig, C., Stingl, K., Pawlik, A., *Profiling of the Helicobacter pylori redox switch HP1021 regulon using a multi-omics approach.* **Nature Communications** 14, 6715 (2023), doi.org/10.1038/s41467-023-42364-6,

is correctly characterized in the table below.

Contributor	Description of main tasks
Mateusz Noszka	**Conceptualization:** throughout project implementation. **Methodology:** implementing RNA-seq and ChIP-seq protocols for *Helicobacter pylori*. **Investigation:** conduction the experiments presented in this work, except EMSA assay, LC-MS/MS and NGS (RNA-seq and ChIP-seq) analyses and bioinformatics (both analyses performed on biological material I prepared); co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Formal analysis and Software:** statistical data analysis except for LC-MS/MS and NGS; NGS code modifications under Agnieszka Strzałka supervision. **Visualization**: preparation of all results for publication, including data collection, analysis (in cooperation with co-authors), and preparation of figures and tables - Fig. 1, 2, 3, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, Tab. S1, S2, Supplementary Data 1 and 2, Source data and Reporting summary. **Data curation:** Supplementary Data 1-2; RNA-seq and ChIP-seq data deposition in ArrayExpress. **Validation:** throughout project implementation. **Writing:** preparing a manuscript draft; reviewing and editing; preparing responses to reviewers. **Funding acquisition:** subproject 0000466 of the EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Agnieszka Strzałka	**Methodology, Formal analysis and Software:** bioinformatic NGS data analysis (RNA-seq and ChIP-seq). **Investigation and Visualization:** code preparation for most NGS and LC-MS/MS plots. **Supervision:** PhD student Mateusz Noszka in bioinformatic analyses. **Validation:** NGS data. **Writing:** original draft of NGS methodology; manuscript review and editing.
Jakub Muraszko	**Investigation:** EMSA assay; co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Writing:** manuscript review and editing.
Rafał Kolenda	**Formal analysis, Software, Methodology and Visualization:** code preparation for COG plots. **Writing:** manuscript review and editing.
Chen Meng	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Validation:** LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing.
Christina Ludwig	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing. **Validation:** LC-MS/MS data. **Data curation:** LC-MS/MS data deposition in ProteomeXchange Consortium. **Funding acquisition:** EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Kerstin Stingl	**Conceptualization, Methodology and Resources:** support in the DNA uptake assay. **Writing:** manuscript review and editing.
Anna Pawlik	**Conceptualization:** initial project proposal and throughout project implementation. **Visualization:** Supplementary Figure S12 and Supplementary

	Data 3. **Validation and Formal Analysis**: throughout project implementation. **Data curation:** Supplementary Data 3. **Supervision:** PhD student Mateusz Noszka. **Writing**: preparing a manuscript draft, reviewing and editing, and preparing responses to reviewers. **Funding acquisition and Project administration:** OPUS 17, Project Number 2019/33/B/NZ6/01648, founded by the National Science Centre, Poland.

.....

(co-author signature)

10.07.2024

Declaration

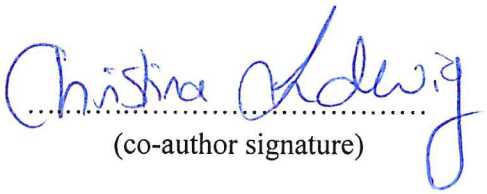
I, Christina Ludwig, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Kolenda, R., Meng, C., Ludwig, C., Stingl, K., Pawlik, A., *Profiling of the Helicobacter pylori redox switch HP1021 regulon using a multi-omics approach.* **Nature Communications** 14, 6715 (2023), doi.org/10.1038/s41467-023-42364-6,

is correctly characterized in the table below.

Contributor	Description of main tasks
Mateusz Noszka	**Conceptualization:** throughout project implementation. **Methodology:** implementing RNA-seq and ChIP-seq protocols for *Helicobacter pylori*. **Investigation:** conduction the experiments presented in this work, except EMSA assay, LC-MS/MS and NGS (RNA-seq and ChIP-seq) analyses and bioinformatics (both analyses performed on biological material I prepared); co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Formal analysis and Software:** statistical data analysis except for LC-MS/MS and NGS; NGS code modifications under Agnieszka Strzałka supervision. **Visualization**: preparation of all results for publication, including data collection, analysis (in cooperation with co-authors), and preparation of figures and tables - Fig. 1, 2, 3, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, Tab. S1, S2, Supplementary Data 1 and 2, Source data and Reporting summary. **Data curation:** Supplementary Data 1-2; RNA-seq and ChIP-seq data deposition in ArrayExpress. **Validation:** throughout project implementation. **Writing:** preparing a manuscript draft; reviewing and editing; preparing responses to reviewers. **Funding acquisition:** subproject 0000466 of the EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Agnieszka Strzałka	**Methodology, Formal analysis and Software:** bioinformatic NGS data analysis (RNA-seq and ChIP-seq). **Investigation and Visualization:** code preparation for most NGS and LC-MS/MS plots. **Supervision:** PhD student Mateusz Noszka in bioinformatic analyses. **Validation:** NGS data. **Writing:** original draft of NGS methodology; manuscript review and editing.
Jakub Muraszko	**Investigation:** EMSA assay; co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Writing:** manuscript review and editing.
Rafał Kolenda	**Formal analysis, Software, Methodology and Visualization:** code preparation for COG plots. **Writing:** manuscript review and editing.
Chen Meng	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Validation:** LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing.
Christina Ludwig	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing. **Validation:** LC-MS/MS data. **Data curation:** LC-MS/MS data deposition in ProteomeXchange Consortium. **Funding acquisition:** EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Kerstin Stingl	**Conceptualization, Methodology and Resources:** support in the DNA uptake assay. **Writing:** manuscript review and editing.
Anna Pawlik	**Conceptualization:** initial project proposal and throughout project implementation. **Visualization:** Supplementary Figure S12 and Supplementary

	Data 3. **Validation and Formal Analysis**: throughout project implementation. **Data curation:** Supplementary Data 3. **Supervision:** PhD student Mateusz Noszka. **Writing**: preparing a manuscript draft, reviewing and editing, and preparing responses to reviewers. **Funding acquisition and Project administration:** OPUS 17, Project Number 2019/33/B/NZ6/01648, founded by the National Science Centre, Poland.



(co-author signature)

10.07.2024

Declaration

I, Kerstin Stingl, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Kolenda, R., Meng, C., Ludwig, C., Stingl, K., Pawlik, A., *Profiling of the Helicobacter pylori redox switch HP1021 regulon using a multi-omics approach.* **Nature Communications** 14, 6715 (2023), doi.org/10.1038/s41467-023-42364-6,

is correctly characterized in the table below.

Contributor	Description of main tasks
Mateusz Noszka	**Conceptualization:** throughout project implementation. **Methodology:** implementing RNA-seq and ChIP-seq protocols for *Helicobacter pylori*. **Investigation:** conduction the experiments presented in this work, except EMSA assay, LC-MS/MS and NGS (RNA-seq and ChIP-seq) analyses and bioinformatics (both analyses performed on biological material I prepared); co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Formal analysis and Software:** statistical data analysis except for LC-MS/MS and NGS; NGS code modifications under Agnieszka Strzałka supervision. **Visualization**: preparation of all results for publication, including data collection, analysis (in cooperation with co-authors), and preparation of figures and tables - Fig. 1, 2, 3, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, Tab. S1, S2, Supplementary Data 1 and 2, Source data and Reporting summary. **Data curation:** Supplementary Data 1-2; RNA-seq and ChIP-seq data deposition in ArrayExpress. **Validation:** throughout project implementation. **Writing:** preparing a manuscript draft; reviewing and editing; preparing responses to reviewers. **Funding acquisition:** subproject 0000466 of the EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Agnieszka Strzałka	**Methodology, Formal analysis and Software:** bioinformatic NGS data analysis (RNA-seq and ChIP-seq). **Investigation and Visualization:** code preparation for most NGS and LC-MS/MS plots. **Supervision:** PhD student Mateusz Noszka in bioinformatic analyses. **Validation:** NGS data. **Writing:** original draft of NGS methodology; manuscript review and editing.
Jakub Muraszko	**Investigation:** EMSA assay; co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Writing:** manuscript review and editing.
Rafał Kolenda	**Formal analysis, Software, Methodology and Visualization:** code preparation for COG plots. **Writing:** manuscript review and editing.
Chen Meng	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Validation:** LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing.
Christina Ludwig	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing. **Validation:** LC-MS/MS data. **Data curation:** LC-MS/MS data deposition in ProteomeXchange Consortium. **Funding acquisition:** EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Kerstin Stingl	**Conceptualization, Methodology and Resources:** support in the DNA uptake assay. **Writing:** manuscript review and editing.
Anna Pawlik	**Conceptualization:** initial project proposal and throughout project implementation. **Visualization:** Supplementary Figure S12 and Supplementary

	Data 3. **Validation and Formal Analysis**: throughout project implementation. **Data curation:** Supplementary Data 3. **Supervision:** PhD student Mateusz Noszka. **Writing**: preparing a manuscript draft, reviewing and editing, and preparing responses to reviewers. **Funding acquisition and Project administration:** OPUS 17, Project Number 2019/33/B/NZ6/01648, founded by the National Science Centre, Poland.

K. Sy

.....

(co-author signature)

10.07.2024

Declaration

I, Anna Pawlik, hereby declare that my contribution to the following manuscript:

Noszka, M., Strzałka, A., Muraszko, J., Kolenda, R., Meng, C., Ludwig, C., Stingl, K., Pawlik, A., *Profiling of the Helicobacter pylori redox switch HP1021 regulon using a multi-omics approach.* **Nature Communications** 14, 6715 (2023), doi.org/10.1038/s41467-023-42364-6,

is correctly characterized in the table below.

Contributor	Description of main tasks
Mateusz Noszka	**Conceptualization:** throughout project implementation. **Methodology:** implementing RNA-seq and ChIP-seq protocols for *Helicobacter pylori*. **Investigation:** conduction the experiments presented in this work, except EMSA assay, LC-MS/MS and NGS (RNA-seq and ChIP-seq) analyses and bioinformatics (both analyses performed on biological material I prepared); co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Formal analysis and Software:** statistical data analysis except for LC-MS/MS and NGS; NGS code modifications under Agnieszka Strzałka supervision. **Visualization**: preparation of all results for publication, including data collection, analysis (in cooperation with co-authors), and preparation of figures and tables - Fig. 1, 2, 3, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, Tab. S1, S2, Supplementary Data 1 and 2, Source data and Reporting summary. **Data curation:** Supplementary Data 1-2; RNA-seq and ChIP-seq data deposition in ArrayExpress. **Validation:** throughout project implementation. **Writing:** preparing a manuscript draft; reviewing and editing; preparing responses to reviewers. **Funding acquisition:** subproject 0000466 of the EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Agnieszka Strzałka	**Methodology, Formal analysis and Software:** bioinformatic NGS data analysis (RNA-seq and ChIP-seq). **Investigation and Visualization:** code preparation for most NGS and LC-MS/MS plots. **Supervision:** PhD student Mateusz Noszka in bioinformatic analyses. **Validation:** NGS data. **Writing:** original draft of NGS methodology; manuscript review and editing.
Jakub Muraszko	**Investigation:** EMSA assay; co-investigation in the fluorescent DNA uptake assay of *H. pylori* N6. **Writing:** manuscript review and editing.
Rafał Kolenda	**Formal analysis, Software, Methodology and Visualization:** code preparation for COG plots. **Writing:** manuscript review and editing.
Chen Meng	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Validation:** LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing.
Christina Ludwig	**Methodology:** bioinformatic analysis of the LC-MS/MS data. **Writing:** methodology writing; manuscript review and editing. **Validation:** LC-MS/MS data. **Data curation:** LC-MS/MS data deposition in ProteomeXchange Consortium. **Funding acquisition:** EPIC-XS, Project Number 823839, funded by the Horizon 2020 Program of the European Union.
Kerstin Stingl	**Conceptualization, Methodology and Resources:** support in the DNA uptake assay. **Writing:** manuscript review and editing.
Anna Pawlik	**Conceptualization:** initial project proposal and throughout project implementation. **Visualization:** Supplementary Figure S12 and Supplementary

	Data 3. **Validation and Formal Analysis**: throughout project implementation. **Data curation:** Supplementary Data 3. **Supervision:** PhD student Mateusz Noszka. **Writing**: preparing a manuscript draft, reviewing and editing, and preparing responses to reviewers. **Funding acquisition and Project administration:** OPUS 17, Project Number 2019/33/B/NZ6/01648, founded by the National Science Centre, Poland.

.....Pawy.....

(co-author signature)

2.2. Publication II

Mateusz Noszka, Agnieszka Strzałka, Jakub Muraszko, Dirk Hofreuter, Miriam Abele, Christina Ludwig, Kerstin Stingl, Anna Zawilak-Pawlik, CemR atypical response regulator impacts energy conversion in *Campylobacteria*. mSystems, 9, 8, 2024. IF_{2023} 5, pkt MNiSW 140.

AMERICAN SOCIETY FOR MICROBIOLOGY | mSystems

| Open Peer Review | Systems Biology | Research Article

CemR atypical response regulator impacts energy conversion in *Campylobacteria*

Mateusz Noszka,[1] Agnieszka Strzałka,[2] Jakub Muraszko,[1] Dirk Hofreuter,[3] Miriam Abele,[4] Christina Ludwig,[4] Kerstin Stingl,[5] Anna Zawilak-Pawlik[1]

AUTHOR AFFILIATIONS See affiliation list on p. 22.

ABSTRACT *Campylobacter jejuni* and *Arcobacter butzleri* are microaerobic food-borne human gastrointestinal pathogens that mainly cause diarrheal disease. These related species of the *Campylobacteria* class face variable atmospheric environments during infection and transmission, ranging from nearly anaerobic to aerobic conditions. Consequently, their lifestyles require that both pathogens need to adjust their metabolism and respiration to the changing oxygen concentrations of the colonization sites. Our transcriptomic and proteomic studies revealed that *C. jejuni* and *A. butzleri,* lacking a *Campylobacteria*-specific regulatory protein, *C. jejuni* Cj1608, or a homolog, *A. butzleri* Abu0127, are unable to reprogram tricarboxylic acid cycle or respiration pathways, respectively, to produce ATP efficiently and, in consequence, adjust growth to changing oxygen supply. We propose that these *Campylobacteria* energy and metabolism regulators (CemRs) are long-sought transcription factors controlling the metabolic shift related to oxygen availability, essential for these bacteria's survival and adaptation to the niches they inhabit. Besides their significant universal role in *Campylobacteria*, CemRs, as pleiotropic regulators, control the transcription of many genes, often specific to the species, under microaerophilic conditions and in response to oxidative stress.

IMPORTANCE *C. jejuni* and *A. butzleri* are closely related pathogens that infect the human gastrointestinal tract. In order to infect humans successfully, they need to change their metabolism as nutrient and respiratory conditions change. A regulator called CemR has been identified, which helps them adapt their metabolism to changing conditions, particularly oxygen availability in the gastrointestinal tract so that they can produce enough energy for survival and spread. Without CemR, these bacteria, as well as a related species, *Helicobacter pylori*, produce less energy, grow more slowly, or, in the case of *C. jejuni*, do not grow at all. Furthermore, CemR is a global regulator that controls the synthesis of many genes in each species, potentially allowing them to adapt to their ecological niches as well as establish infection. Therefore, the identification of CemR opens new possibilities for studying the pathogenicity of *C. jejuni* and *A. butzleri*.

KEYWORDS *Campylobacter jejuni*, *Arcobacter butzleri*, carbon metabolism, respiration, transcription factors, proteomics, transcriptomics, oxidative stress, *Helicobacter pylori*, signal transduction

Campylobacter jejuni and *Arcobacter butzleri* are Gram-negative, microaerobic bacteria that belong to the *Campylobacteria* class (1). *C. jejuni* is a commensal bacterium of the gastrointestinal tracts of wildlife and domestic animals. However, in humans, *C. jejuni* is the leading cause of food-borne bacterial diarrheal disease (2). Also, it causes autoimmune neurological diseases such as Guillian-Barré and Miller-Fisher syndromes (3). *A. butzleri* is found in many ecological niches, such as environmental water and animals. Still, in humans, *A. butzleri* causes diarrhea, enteritis, and bacteremia (2). Due to the wide distribution of the

Editor Jack A. Gilbert, University of California San Diego, La Jolla, California, USA

Address correspondence to Anna Zawilak-Pawlik, anna.pawlik@hirszfeld.pl.

The authors declare no conflict of interest.

See the funding table on p. 22.

Received 9 June 2024
Accepted 12 June 2024
Published 9 July 2024

two species and relatively high resistance to environmental conditions (4) and antibiotics (5, 6), both species pose a severe threat to human health.

To adapt to different ecological niches and decide whether conditions are optimal for reproduction and transmission, *C. jejuni* and *A. butzleri* encode numerous regulatory proteins. According to the MiST database (7), *C. jejuni* NCTC 11168 [1.64 Mbp; 1,643 coding sequences (8)] and *A. butzleri* RM4018 [2.34 Mbp; 2,259 coding sequences (9)] encode 56 and 218 signal transduction proteins, respectively. While *A. butzleri* regulatory systems have been hardly studied (10), the *C. jejuni* signal transduction systems have been partially deciphered (11–15). Particular interest was directed to studying processes related to the *C. jejuni* colonization and focused on factors controlling the expression of genes involved in such processes as host adaptation and niche detection (BumSR and DccSR), oxidative stress (e.g., CosR and PerR), metabolism, and respiration [e.g., BumSR, RacSR, CosR, CsrA, LysR (Cj1000), and CprSR] (11, 13–15). Notably, aerobic or microaerophilic respiration, in which oxygen is used as a terminal electron acceptor, is far more efficient in supplying energy than anaerobic respiration or fermentation (16). On the other hand, oxygen-dependent respiration is a source of reactive oxygen species (ROS) (17). *C. jejuni* requires oxygen for growth but, unlike facultative aerobic *A. butzleri*, is sensitive to aerobic conditions faced during transmission. In addition, *C. jejuni* and *A. butzleri* may encounter conditions deficient in oxygen in the intestine or during intracellular persistence (18). Thus, *C. jejuni* and *A. butzleri* must adjust their metabolism and respiration as oxygen concentrations change at colonization sites to get sufficient energy while maintaining redox homeostasis. However, no orthologs of energy or redox metabolism regulators such as gammaproteobacterial ArcA, FNR, or NarP are found in *C. jejuni* (14, 19–21).

The roles of many *C. jejuni* and most *A. butzleri* regulators have still not been explored. One such regulator is the *C. jejuni* NCTC 11168 Cj1608 orphan response regulator, which is homologous to uncharacterized *A. butzleri* RM4018 Abu0127. These regulators are homologous to *Helicobacter pylori* 26695 HP1021 and are conserved across most *Campylobacteria* class species, constituting most of the *Campylobacterota* phylum.

The *H. pylori* HP1021 response regulator is one of 28 signal transduction proteins encoded by *H. pylori* 26695 (7, 22). HP1021 interacts with the origin of chromosome replication region (*oriC*) *in vitro*, probably participating in the control of the initiation of *H. pylori* chromosome replication (23). HP1021 acts as a redox switch protein, i.e., senses redox imbalance and transmits the signal and triggers the cells' response (24). The HP1021 regulon, initially determined in *H. pylori* 26695 by microarray analyses (25), has been updated in *H. pylori* N6 using a multi-omics approach (26). HP1021 influences the transcription of almost 30% of all *H. pylori* N6 genes of different cellular categories; the transcription of most of these genes is related to response to oxidative stress. HP1021 directly controls typical ROS response pathways and less canonical ones, such as central carbohydrate metabolism. The level of ATP and the growth rate of the knock-out *H. pylori* ΔHP1021 are lower than in the wild-type strain, which is possibly due to reduced transcription of many tricarboxylic acid cycle (TCA) genes and/or increased ATP consumption in catabolic processes in ΔHP1021 compared to the wild-type strain. Thus, HP1021, among many cellular processes, probably controls *H. pylori* metabolic fluxes to maintain the balance between anabolic and catabolic reactions, possibly for efficient oxidative stress response (26).

In this work, we focused on the two hardly characterized regulatory proteins of the microaerobic *C. jejuni* NCTC 11168 and the facultatively aerobic *A. butzleri* RM4018, Cj1608, and Abu0127, respectively. To get insight into the function of these proteins, we constructed mutants lacking these regulators and looked at transcriptomic and proteomic changes under optimal growth and oxidative stress conditions. Our results indicate that Cj1608 and Abu0127, which we named *Campylobacteria* energy and metabolism regulators (CemRs), support energy conservation in bacterial cells by controlling metabolic and respiration pathways in response to oxygen availability.

RESULTS

Influence of the Cj1608 regulator on *C. jejuni* gene and protein expression

Cj1608 has been hardly characterized thus far. It is only known that it interacts *in vitro* with the *C. jejuni oriC* region and the promoter of the *lctP* lactate transporter, probably participating in the control of the initiation of the *C. jejuni* chromosome replication and lactate metabolism (27, 28). Therefore, to elucidate the role of the Cj1608 regulator in controlling *C. jejuni* gene expression and oxidative stress response, we performed transcriptome analysis [RNA sequencing (RNA-seq)] of the *C. jejuni* NCTC 11168 wild-type (CJ) and deletion mutant (ΔCj1608) strains under microaerobic growth (CJ, ΔCj1608) and during paraquat-induced oxidative stress (CJ_S, $\Delta Cj1608_S$) (Fig. S1A).

A comparison of *C. jejuni* ΔCj1608 and CJ transcriptomes revealed 380 differentially transcribed genes (Fig. 1A and B; Fig. S2A; S1 Data). The paraquat stress affected the transcription of genes in wild type and ΔCj1608 strains (232 and 123 genes, respectively) (Fig. 1B; Fig. S2B and C). The transcription of 44 genes was similarly induced or repressed in CJ_S and $\Delta Cj1608_S$ cells (Fig. S2D, red dots). Thus, these genes responded to oxidative stress, but other or additional factors than Cj1608 controlled them. Using ClusterProfiler (29), we performed a Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis to identify processes affected most by the lack of Cj1608 or under stress. In the ΔCj1608 strain, three KEGG groups were activated (nitrogen metabolism, ribosomes, and aminoacyl-tRNA-biosynthesis). One KEGG group was suppressed, namely, the TCA cycle (Fig. 1C). In the CJ strain under stress conditions, six KEGG groups were suppressed (e.g., oxidative phosphorylation), while four KEGG groups were activated (e.g., carbon metabolism).

Since post-transcriptional regulation is common in bacteria, including species closely related to *C. jejuni* (30, 31), a proteomics approach [liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)] was applied to detect proteins whose levels changed between analyzed strains or conditions. A total of 1,170 proteins were detected (S1 Data); however, 173 proteins encoded by genes whose transcription changed in ΔCj1608 were not detected by MS (S1 Data). The comparison between proteomes of ΔCj1608 and CJ strains under microaerobic conditions revealed different levels of 156 proteins (Fig. S4A; red, yellow, and green dots). The transcriptomic and proteomic data correlated strongly (Fig. 1D, red dots; Fig. S5A). However, the differences between transcription and translation patterns were observed for many genes, suggesting a post-transcriptional regulation of *C. jejuni* gene expression (Fig. 1D; blue, yellow, and green dots). Under oxidative stress, the expression of only four genes changed, namely, the expression of ferrochelatase HemH (Cj0503c) was downregulated in the CJ_S cells; at the same time, the levels of three proteins were upregulated [the catalase KatA (Cj1385), the atypical hemin-binding protein Cj1386, mediating the heme trafficking to KatA (32), and the periplasmic protein Cj0200c] (Fig. S4B and C). The small number of proteins whose expression was altered under oxidative stress confirmed the known phenomenon of ceasing protein synthesis by bacteria under oxidative stress (26, 33, 34). The ClusterProfiler KEGG enrichment analysis revealed that genes of two KEGG groups, nitrogen metabolism and ribosome, were activated in the ΔCj1608 strain in comparison to CJ, while the genes of three KEGG groups were suppressed (TCA cycle, ABC transporters, and carbon metabolism) (Fig. 1E). It should be noted that the nitrogen metabolism group includes proteins such as GlnA and GltBD, which are involved in glutamate metabolism and TCA cycle (35), whose levels increased more than 10-fold, and Nap and Nrf complexes, constituting an electron transport chain (ETC) system (see below).

To conclude, the transcriptomic and proteomic data revealed that Cj1608 impacts the transcription of 585 genes and the expression of 156 proteins in *C. jejuni* NCTC 11168, with the TCA and nitrogen metabolism KEGG groups most affected.

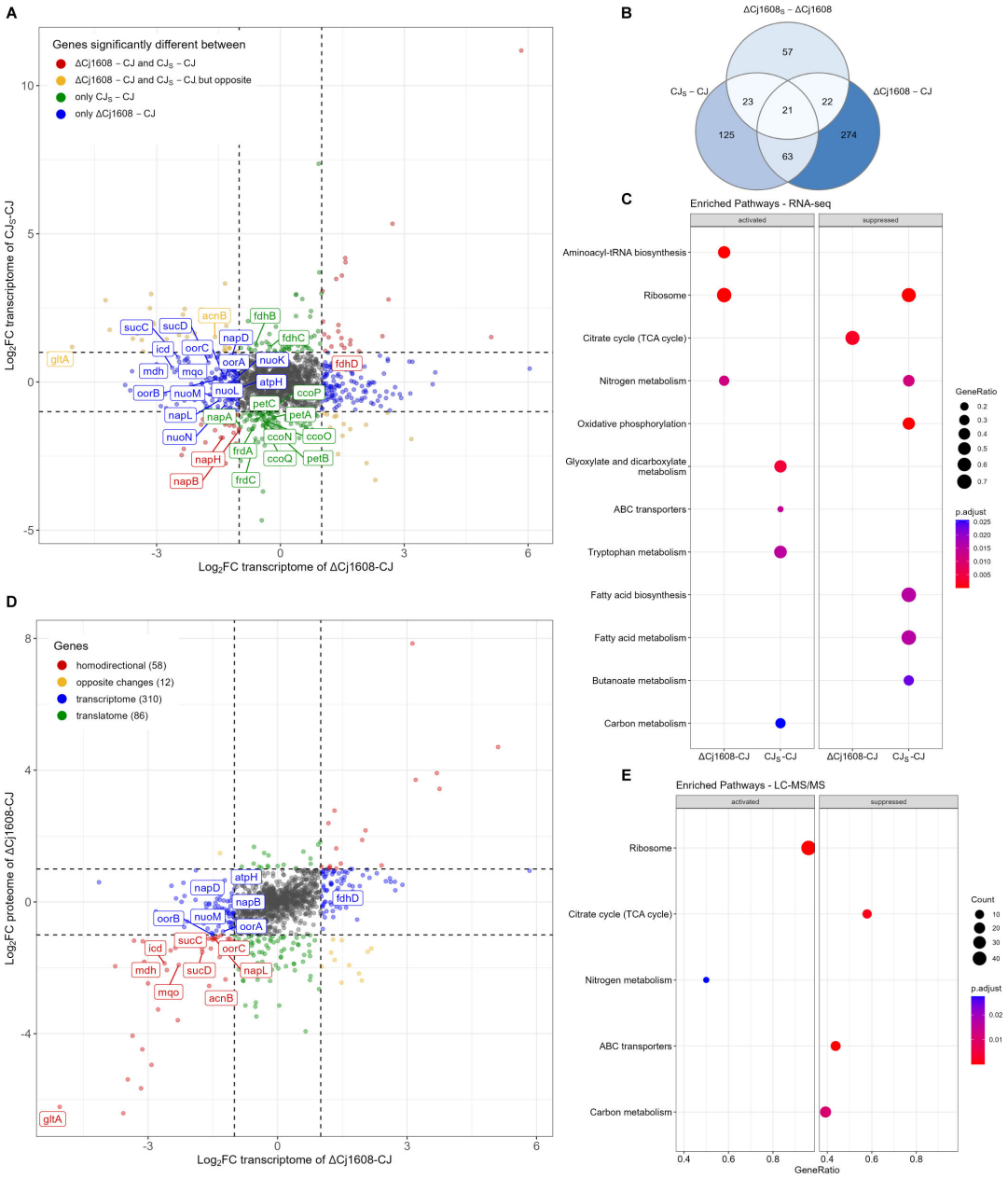


FIG 1 RNA-seq and LC-MS/MS analyses of *C. jejuni* gene expression controlled by Cj1608. (A) The comparison of gene transcription in the CJ, CJ_S, and Cj1608 knock-out mutant (ΔCj1608) cells revealed by RNA-seq. Genes whose transcription significantly changed ($|\log_2 FC| \geq 1$, FDR < 0.05) are depicted by colored dots (see the legend in the figure). The genes named on the graph correspond to the citric acid cycle and electron transport chain. (B) The Venn diagram presents the number of differentially transcribed genes in the analyzed strains and conditions. (C) ClusterProfiler protein enrichment plot showing activation or suppression of KEGG groups in the analyzed strains. (D) The correlation between gene expression in ΔCj1608 and CJ cells at the transcription and proteome levels. Red dots represent homodirectional genes up-regulated or down-regulated at the transcriptome and proteome levels. Yellow dots represent opposite changes. Blue dots represent genes that changed only at the transcription level, while green dots represent genes that changed only at the proteome level. Numbers of differentially expressed genes in the indicated strains and conditions are given in parentheses. The proteins named on the graph correspond to the citric acid cycle and electron transport chain. (E) ClusterProfiler protein enrichment plot showing activation or suppression of KEGG groups in the analyzed strains. (A and D) Values outside the black dashed lines indicate a change in the expression of $|\log_2 FC| \geq 1$. Gray dots correspond to genes whose transcription was not changed ($|\log_2 FC| < 1$). CJ, *C. jejuni* wild type; CJ_S, stressed wild type; FC, fold change; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry.

Influence of Abu0127 on *A. butzleri* gene and protein expression

To elucidate the role of the Abu0127 regulator in controlling gene expression and oxidative stress response of *A. butzleri*, we performed RNA-seq and LC-MS/MS analyses similar to those for *C. jejuni*. We analyzed gene expression in *A. butzleri* RM4018 wild-type (AB) and deletion mutant (ΔAbu0127) strains; paraquat was used to induce oxidative stress (AB_S, $\Delta Abu0127_S$) [Fig. S1B (36)].

Comparison of *A. butzleri* ΔAbu127 and AB transcriptomes revealed 779 differentially transcribed genes (Fig. 2A and B; Fig. S3A; S2 Data). The paraquat stress affected the transcription of genes in the wild type and, to a much lesser extent, in the ΔCj1608 strain (290 and 14 genes, respectively) (Fig. 2B; Fig. S3B and C). Of these 14 differently transcribed genes in the ΔCj1608 strain, 12 were also induced in AB_S cells (Fig. S3D, red dots). Thus, these genes responded to oxidative stress but were controlled by factors other than or additional to Abu0127. KEGG enrichment analysis revealed that in the ΔAbu0127 strain, 2 KEGG groups were activated, ABC transporters and sulfur metabolism, while 13 groups were suppressed (e.g., oxidative phosphorylation and TCA cycle) (Fig. 2C). In the AB strain under stress conditions, seven KEGG groups were suppressed (e.g., oxidative phosphorylation and citrate cycle), while six groups were activated (e.g., sulfur metabolism).

As in our *C. jejuni* study, a proteomic approach was used to detect proteins whose levels changed between the strains and conditions analyzed. A total of 1,586 *A. butzleri* proteins were detected (S2 Data). However, proteomics did not detect 224 proteins encoded by genes whose transcription changed in ΔAbu0127 (S2 Data). The comparison between proteomes of ΔAbu0127 and AB strains under microaerobic conditions revealed 124 differentially expressed proteins (Fig. 2D; Fig. S4D, red, yellow, and green dots). As in *C. jejuni* studies, the transcriptomics and proteomics data correlated (Fig. 2D, red dots; Fig. S5B;); however, in cases of many genes, post-transcriptional modifications probably affected the final protein levels in the *A. butzleri* cell (Fig. 2D; blue, yellow, and green dots). Under paraquat stress, the level of only one protein changed; namely, Abu0530, of unknown function, was produced at a higher level in AB_S cells compared to AB cells (Fig. S4E and F). Thus, *A. butzleri*, like *C. jejuni*, ceased protein translation upon oxidative stress, which is also typical for other bacterial species (37). The ClusterProfiler KEGG enrichment analysis revealed that genes of one KEGG group, sulfur metabolism, were activated in the ΔAbu0127 strain compared to AB. In contrast, the genes of three KEGG groups were suppressed (oxidative phosphorylation, taurine and hypotaurine metabolism, and ribosome) (Fig. 2E).

To conclude, the results of transcriptomic and proteomic analyses indicated that Abu0127 affects the transcription of 904 genes and the expression of 124 proteins in *A. butzleri* RM4018, with the oxidative phosphorylation KEGG group most affected.

Cj1608 and Abu0127 are involved in the regulation of energy production and conversion

The transcriptome and proteome analyses of both species revealed that the energy production and conversion processes, such as the TCA cycle and oxidative phosphorylation, are putatively controlled in *C. jejuni* by Cj1608 and in *A. butlzeri* by Abu0127 (S1 Data, S2 Data and S3 Data). In *C. jejuni* ΔCj1608, the protein levels of all but two TCA enzymes, fumarate reductase FrdABC and fumarate hydratase FumC, decreased, with the citrate synthase GltA protein level reduced by as much as 70-fold (Fig. 3A; Fig. S6; the level of oxoglutarate-acceptor oxidoreductase Oor was reduced by 1.7- to 2.0-fold). Many genes encoding nutrient importers and downstream processing enzymes were downregulated in *C. jejuni* ΔCj1608 (e.g., amino acid ABC transporter permease Peb, fumarate importer DcuAB, L-lactate permease LctP, and lactate utilization proteins Lut). However, DctA succinate/aspartate importer and the enzymes glutamine synthetase GlnA and glutamate synthase small subunit GltB involved in glutamine and glutamate synthesis were upregulated (Fig. 3A). The levels of many proteins forming ETC complexes

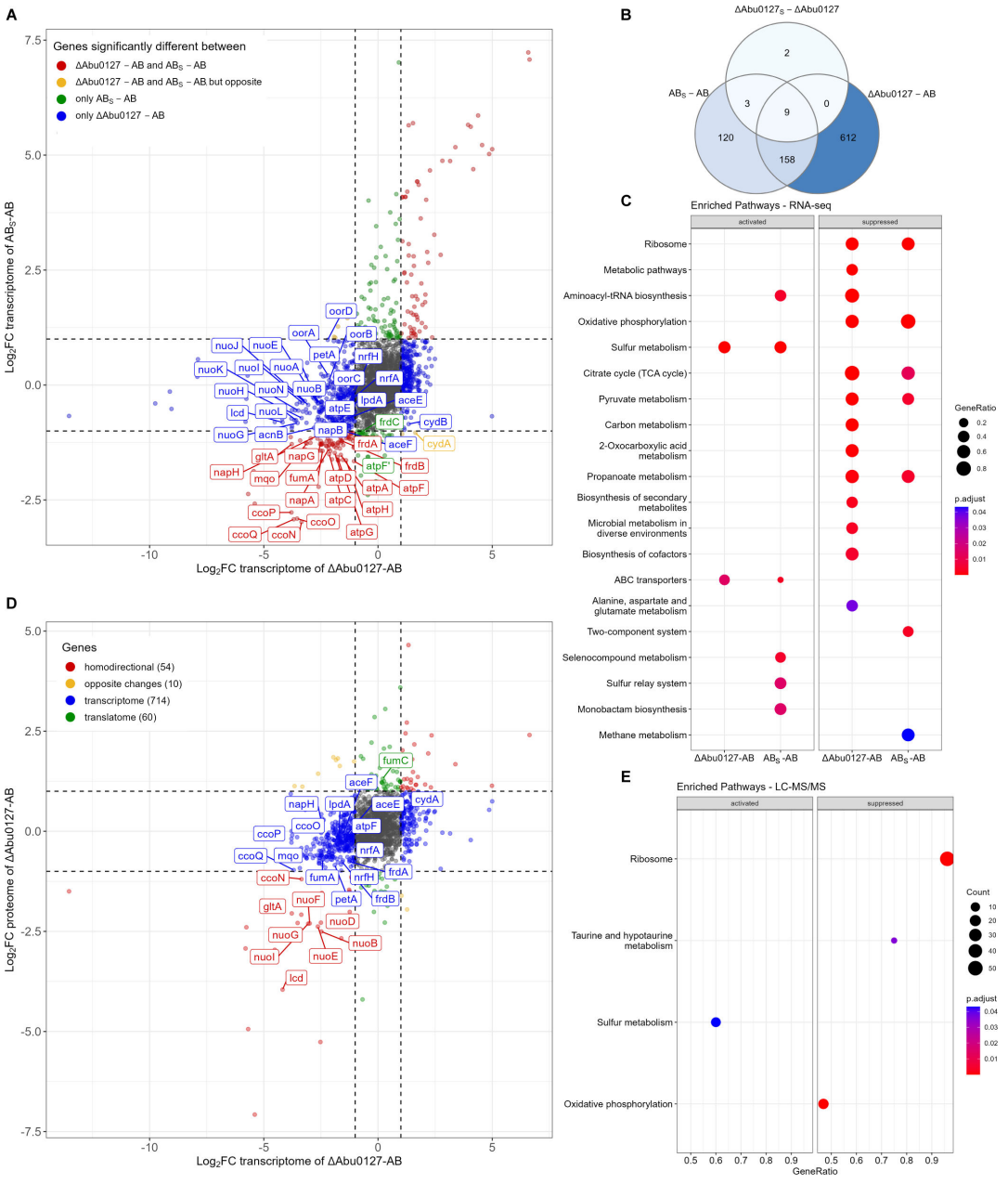


FIG 2 RNA-seq and LC-MS/MS analyses of *A. butzleri* gene expression controlled by Abu0127. (A) The comparison of gene transcription in the AB, AB$_S$, and Abu0127 knock-out mutant (ΔAbu0127) cells revealed by RNA-seq. Genes whose transcription significantly changed ($|\log_2 FC| \geq 1$; FDR < 0.05) are depicted by colored dots (see the legend in the figure). The genes named on the graph correspond to the citric acid cycle and electron transport chain. (B) The Venn diagram presents the number of differentially transcribed genes in the analyzed strains and conditions. (C) ClusterProfiler protein enrichment plot showing activation or suppression of KEGG groups in the analyzed strains. (D) The correlation between gene expression of ΔAbu0127 and AB cells at the transcription and proteome levels. Red dots represent homodirectional genes up-regulated or down-regulated at the transcriptome and proteome levels. Yellow dots represent opposite changes. Blue dots represent genes that changed only at the transcription level, while green dots represent genes that changed only at the proteome level. Numbers of differentially expressed genes in the indicated strains and conditions are given in parentheses. The proteins named on the graph correspond to the citric acid cycle and electron transport chain. (E) ClusterProfiler protein enrichment plot showing activation or suppression of KEGG groups in the analyzed strains. (A and D) Values outside the black dashed lines indicate a change in the expression of $|\log_2 FC| \geq 1$. Gray dots correspond to genes whose transcription was not changed ($|\log_2 FC| < 1$). AB, *A. butzleri* wild type; AB$_S$, stressed wild type, FC, fold change; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry.

were also downregulated, including subunits of NADH-quinone oxidoreductase Nuo and cytochromes (Fig. 3B). In *A. butzleri* ΔAbu0127, the levels of GltA and isocitrate

dehydrogenase Icd proteins were reduced (Fig. 3C; Fig. S7C). As in *C. jejuni*, the levels of many proteins forming ETC complexes decreased, with Nuo complex subunits being the most highly downregulated (Fig. 3D; Fig. S7A and B).

Moreover, a functional analysis annotation with eggNOG-mapper indicated the Clusters of Orthologous Groups (COGs) of differentially expressed genes and proteins. In both species, the most variable groups belonged to two categories, namely, energy production and conservation (C) and translation, ribosomal structure, and biogenesis (J) (Fig. 4; the category of unknown genes was excluded from the graph, S1 Data and S2 Data). Many genes and proteins suppressed in both species belong to the energy production and conversion group.

These comprehensive data suggest that mutants of both species produce less energy due to the inhibition of the TCA cycle and oxidative phosphorylation. Therefore, we decided to investigate these processes in more detail and analyze how the lack of Cj1608 and Abu0127 proteins influences energy production and conversion in *C. jejuni* and *A. butzleri*, respectively.

Cj1608 controls *gltA* expression, ATP level, and growth in response to O_2 supply

To analyze the influence of Cj1608 on *C. jejuni* growth, we compared the growth of wild-type CJ, knock-out ΔCj1608, and complementation (C_{Cj1608}) strains (Fig. 5A). It should be noted that unless an accessory ETC electron donor and proton motive force generator H_2 was supplied in the microaerobic gas mixture (4% H_2; see Materials and Methods) [see also reference (38)], we could not obtain the ΔCj1608 mutant strain of *C. jejuni* NCTC 11168. When *C. jejuni* was cultured microaerobically in the presence of H_2, the growth of *C. jejuni* ΔCj1608 was slower [i.e., the generation time (G) was higher] than the CJ and C_{Cj1608} strains (Fig. 5A). The ΔCj1608 culture entered the stationary phase at optical density (OD) like that of CJ and C_{Cj1608} strains (Fig. 5A). Under microaerobic conditions without H_2, the CJ and C_{Cj1608} strains grew similarly to conditions with H_2, albeit reaching lower OD_{600} at the stationary phase than with H_2 (Fig. 5A). In contrast, the ΔCj1608 strain almost did not grow without H_2, confirming that its growth strictly depended on hydrogen. The ATP analysis indicated that the relative energy levels corresponded to the bacterial growth rates. The ATP level of the CJ strain under microaerobic growth without H_2 was assumed to be 100% (Fig. 5B). The ATP level in CJ and C_{Cj1608} strains was constant, regardless of the presence or absence of H_2 (85%–100%). Without H_2, the ATP level in the ΔCj1608 strain dropped to 25% ± 4% of the ATP level in the CJ strain. In the presence of H_2, the level of ATP in ΔCj1608 cells increased to 53% ± 8% compared to that of the CJ strain. These results indicated that the availability of additional electrons and proton motive force derived from hydrogen (38), an alternative to those produced by the TCA cycle and used in oxidative phosphorylation, enabled ΔCj1608 cells to produce more energy and multiply more efficiently.

Next, we analyzed whether Cj1608 directly affects the TCA cycle efficiency, as suggested by hydrogen boost analysis. We studied the expression of *gltA* since the expression of this gene was severely downregulated in the ΔCj1608 strain (Fig. S8A and B; S3 Data). GltA is the first enzyme in the TCA cycle whose activity impacts the flow of substrates through the TCA cycle and energy production via NADH/FADH/menaquinone cofactors (Fig. 3A). It was previously shown that *gltA* transcription depends on the O_2 concentration, being lower at 1.88% O_2 and higher at 7.5% O_2, which helps *C. jejuni* optimize energy production and expense during different oxygen availability as the electron acceptor (39). Here, we confirmed the specific interaction of the Cj1608 protein with the *gltA* promoter region *in vivo* by chromatin immunoprecipitation (ChIP) and *in vitro* by electrophoretic mobility shift assay (EMSA) (Fig. 5C and D); Cj1608 did not interact with a control *C. jejuni recA* region. Next, we used reverse transcription quantitative PCR (RT-qPCR) to analyze *gltA* transcription under oxidative stress triggered by paraquat or under diverse O_2 availability: reduced 1% O_2, optimal microaerobic 5% O_2, and increased 10% O_2 (see Materials and Methods). Transcription of *gltA* was lower

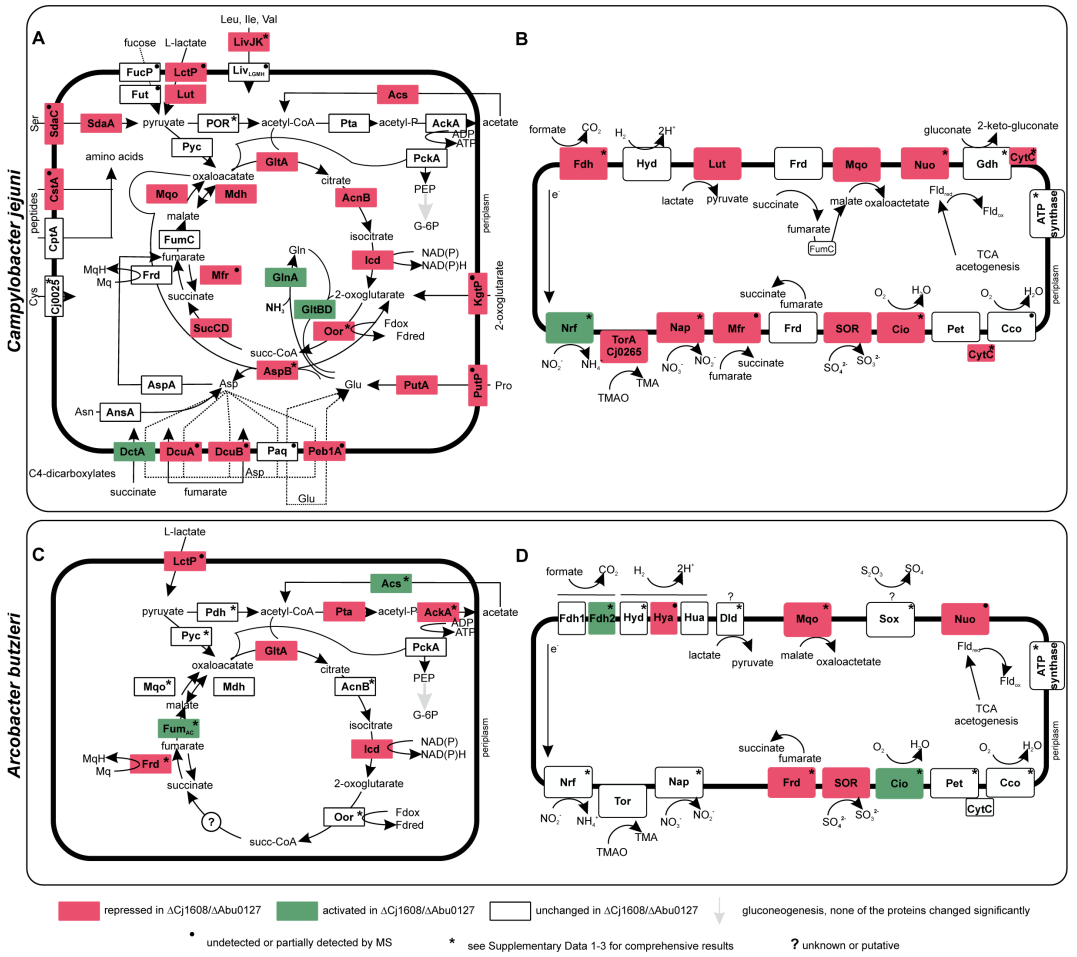


FIG 3 Schematic presentation of *C. jejuni* and *A. butzleri* energy conservation factors whose expression changed in ΔCj1608 and ΔAbu0127, respectively. (A) Regulation of the *C. jejuni* citric acid cycle, the selected associated catabolic pathways, and nutrient transporters. (B) Regulation of *C. jejuni* electron transport chain enzymes. (C) Regulation of the *A. butzleri* tricarboxylic acid cycle selected associated catabolic pathways and nutrient transporters. (D) Regulation of *A. butlzeri* electron transport chain enzymes. (A–D) The increase or decrease in protein abundance in the deletion mutant strains compared to wild-type strains is shown based on the LC-MS/MS data (S3 Data). When MS did not detect the protein but the corresponding transcript level was increased or decreased, the color code depicts RNA-seq results (denoted as •). For details concerning transcriptomic data or complex transcriptome/proteome interpretation (denoted as *), see S1 to S3 Data. Cco, cbb3-type cytochrome c oxidase; Cio, cyanide-insensitive cytochrome bd-like quinol oxidase; Fdh, formate dehydrogenase; Frd, fumarate reductase; G-6P, glucose 6-phosphate; Gdh, gluconate 2-dehydrogenase; Hyd, Hya, and Hua, hydrogenases; Lut, Dld, L-lactate utilization complex; Mfr, methylmenaquinol:fumarate reductase (misannotated as succinate dehydrogenase Sdh); Mqo, malate:quinone oxidoreductase; Nap, nitrate reductase; Nrf, nitrate reductase; Nuo, NADH-quinone oxidoreductase; Oor, 2-oxoglutarate:acceptor oxidoreductase; PEP, phosphoeneoopyruvate; Pet, ubiquinol-cytochrome C reductase; POR, pyruvate:acceptor oxidoreductase; SOR, sulfite:cytochrome c oxidoreductase; SOX, thiosulfate oxidation by SOX system; Tor, SN oxide reductase; "?," unknown enzyme. All genes and enzyme complexes can be found in S3 Data.

in ΔCj1608 than in CJ and C_{Cj1608} strains under optimal conditions [fold change (FC) of 0.14 ± 0.14] and unaffected by paraquat stress, confirming the transcriptomic results (Fig. S8C). Moreover, transcription of *gltA* was upregulated in the CJ and C_{Cj1608} strains at 5% O_2 compared to 1% O_2 (Fig. 5E). However, in the CJ and C_{Cj1608} strains, the *gltA* transcription was downregulated at 10% O_2 compared to 5% O_2 (Fig. 5E), suggesting that the prolonged bacterial growth for 5 h at increased O_2 concentration possibly caused adaptation to higher oxygen level conditions or stress by reducing TCA cycle activity and possible ROS production during increased aerobiosis. However, *gltA* transcription was constant in ΔCj1608 at all O_2 concentrations, indicating that cells could not adjust *gltA* transcription to changing O_2 conditions. Next, we analyzed *C. jejuni* growth and ATP