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PROTON AND POTASSIUM FLUXES IN *ESCHERICHIA COLI* MUTANTS WITH DEFECTS IN SUBUNITS RESPONSIBLE FOR MATURATION OF HYD-1 AND HYD-2 DURING GLUCOSE FERMENTATION

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This study investigates the roles of specific subunits in Hyd-1 and Hyd-2 and their impact on proton and potassium fluxes during fermentation of glucose in *Escherichia coli (E. coli)*. By examining conditions of varying glucose availability, we aimed to uncover how these subunits influence the metabolic adaptability of *E. coli* under fermentative conditions by altering ion fluxes, indicative of their roles in hydrogenase activity and cellular energy metabolism. Notably, *hyaD* and *hyaF* mutants exhibited a 50% increase in DCCD (N, N'-dicyclohexylcarbodiimide)-sensitive proton flux, underlining their critical roles in Hyd-1 activity. Exclusively, the *hybF* mutant demonstrated an increase in DCCD-sensitive flux, suggesting cross-regulation between hydrogenases, particularly underscoring the role of HybF in Hyd-1 activity, resembling the observations with *hyaB* data. Thus, in the presence of low glucose, HybF is presumably less involved in the maturation of Hyd-2. The data for *hybE* and *hybF* strongly imply that under conditions of low glucose growth supplemented with high glucose are crucial for Hyd-2 activity. *HyaD* and *hyaF* mutants exhibited a 50% increase in DCCD-sensitive proton flux, indicating their essential roles in Hyd-1 activity. Taken together, our results suggest that depending on glucose concentration specific subunits may have altered and cross-regulated roles in maturation of Hyd enzymes.

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***Keywords*:** glucose concentration, Hyd-1 and Hyd-2, maturation, proton and potassium fluxes.

Introduction. The hydrogenase (Hyd) enzymes, specifically Hyd-1 and Hyd-2, are of significant interest within the realm of bacterial energy metabolism. These enzymes play a key role in the reversible conversion of molecular hydrogen into protons and electrons [1–3].

Hyd-1 is produced in *E. coli* under anaerobic conditions and mainly works to oxidize hydrogen. It is oxygen-tolerant and works alongside a cytochrome oxidase, suggesting its role is crucial when *E. coli* shifts from anaerobic to aerobic environments [4, 5]. On the other hand, Hyd-2 is sensitive to oxygen and operates under anaerobic conditions to help produce energy by oxidizing hydrogen [6, 7].

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The activity and efficiency of these Hyd enzymes are dependent upon a highly regulated maturational process, involving several subunits crucial for their proper assembly, activation, and integration into the cellular metabolic framework.

Six Hyp proteins (HypABCDEF) play central roles in the maturation, they coordinate the synthesis of ligands, insertion of the nickel ion, assembly of the NiFe cofactor, proteolytic processing, and, in some cases, enzyme translocation, ensuring the activation and proper functioning of [NiFe]-hydrogenases and may act in the maturation of more than one Hyd in the cell [3, 8]. Among these stages, only the proteolytic modification is comprehensively understood at a molecular level [9].

Typically, the genes for the core maturation proteins, known for inserting the nickel-iron center, are expressed separately from the Hyd structural genes. However, there exists a second set of maturation genes that are co-expressed alongside the structural genes within the same transcriptional unit. This arrangement is because their protein products are directly involved in the maturation of the specific isoenzyme they accompany, ensuring each Hyd variant is correctly assembled and functional [3, 10].

Thus, the first three genes in the *hya* operon (*hyaABC*) and the first four genes of the *hyb* operon (*hybOABC*) are responsible for encoding the elements of the membrane-bound catalytic complexes. The subsequent seven genes – HyaD, HyaE, HyaF, HybD, HybE, HybF, and HybG – though not well understood, seem to contribute to the biosynthesis of Hyd rather than directly participating in the hydrogen activation process itself [11]. Based on their function, two classes can be differentiated presently. The first one encompasses endopeptidases like HyaD and HybD, which proteolytically process the precursors of the large subunit from 1 and 2, respectively, after metal incorporation. The second family comprises chaperone-like proteins, which coordinate the assembly and export of periplasmic Hyds via the TAT (the twin-arginine protein)-export system. Representatives in *E. coli* are the *hyaE* and *hybE* gene products that are required for the export of the cofactor-containing heterodimeric Hyd-1 and Hyd-2 [12]. On the other hand, a critical yet often overlooked factor in understanding the functionality of these Hyds is the impact of glucose concentration.

Glucose, as a primary substrate for energy production in *E. coli*, significantly influences the metabolic state of the cell, affecting not just the central metabolic pathways, but also the operation of membrane-bound enzymes and transport systems [1, 13, 14]. The availability of glucose alters the cellular demand for energy and the mechanisms, through which it is produced, thereby directly affecting the activity of Hyd enzymes and associated processes [15–17].

This study focuses on understanding how specific subunits involved in the maturation of Hyd-1 and Hyd-2 influence proton and potassium fluxes during glucose fermentation in *E. coli*. By examining the variations in ion fluxes across membrane during utilization of different glucose concentrations, the research aims to uncover, if and how glucose levels affect the Hyd maturation process. Through this approach, we intend to deepen our understanding of the intricate balance between energy metabolism and ion homeostasis in bacteria, contributing to the broader knowledge of microbial physiology and offering insights into potential biotechnological applications for enhanced energy production.

Materials and Methods.

Bacterial Strains and Growth Conditions. For this study, *E. coli* strains outlined in Table were grown in a peptone-rich buffered medium (pH 7.5) containing 20 $g \cdot L^{-1}$ peptone, 15 $g \cdot L^{-1}$ K_2HPO_4, 1.08 $g \cdot L^{-1}$ KH_2PO_4, 5 $g \cdot L^{-1}$ NaCl) at 37℃. The growth medium was supplemented with glucose at either low (2 $g \cdot L^{-1}$) or high (8 $g \cdot L^{-1}$) concentrations. Cultivation was done in 1000 *mL* Duran bottles, from which oxygen was removed through autoclaving. Subsequently, these bottles were sealed with press-caps and underwent gas exchange, following the protocols as before [17, 18]

Characteristics of E. coli wild type and mutant strains used

Strain	Genetic description	Absent or defective gene product	Reference
BW25113	*lacI^{q}rrnB$_{T14}$ΔlacZ$_{W116}$ hsdR514ΔaraBAD$_{AH33}$ Δrha BAD$_{LD78}$*	wild type	[19]
JW0957	BW25113 Δ*hyaD*	putative hydrogenase 1 maturation protease HyaD	[20]
JW0958	BW25113 Δ*hyaE*	putative HyaA chaperone HyaE	[20]
JW0959	BW25113 Δ*hyaF*	protein HyaF	[20]
JW2961	BW25113 Δ*hybD*	hydrogenase 2 maturation protease HybD	[20]
JW2958	BW25113 Δ*hybG*	hydrogenase maturation factor HybG	[20]
JW2960	BW25113 Δ*hybE*	hydrogenase 2 specific chaperone HybE	[20]
JW5493	BW25113 Δ*hybF*	hydrogenase maturation protein HybF	[20]

Measurement of H^+ and K^+ Fluxes. To assess proton and potassium fluxes, cells were harvested during the late stationary phase. H^+ and K^+ ion fluxes (J_{H^+}, J_{K^+}) were evaluated in cells at the late stationary growth phase, which were subsequently cleansed with distilled water. These fluxes were measured in a 4.5 *mL* Tris-phosphate buffer solution buffer (150 *mM* Tris-phosphate pH 7.5, containing 0.4 *mM* $MgSO_4$, 1 *mM* NaCl, 1 *mM* KCl), employing a pH-selective electrode or a Potassium Combination Ion-Selective Electrode (ISE) for precise potentiometric analysis (HI1131 and HI4114 Hanna Instruments, Portugal). To begin the flux assessment, glucose was introduced into the assays at either a lower or higher concentration, and flux alterations were meticulously documented every minute. Calibration of electrode readings was achieved through the careful titration of the assay medium with either 0.01 *M* HCl or 0.1 *M* KCl solutions [17, 19, 21].

J_{H^+} and J_{K^+}, expressed in millimoles per minute for every 10^9 cells, were determined without the addition of N, N'-dicyclohexylcarbodiimide (DCCD). For the measurement of DCCD-sensitive ion fluxes, cells were treated with 0.2 *mM* DCCD for a duration of 5–7 *min*. The differential between the total ion flux of untreated cells and those treated with DCCD provided the basis for calculating DCCD-sensitive ion fluxes. The ratio of DCCD-sensitive H^+ to K^+ fluxes was determined by dividing the respective DCCD-sensitive flux values [19, 22, 23].

Statistical Analysis. Each data point shown was averaged from independent triplicate cultures presented as mean ±SD. A p-value of less than 0.05 was considered significant. Data were visualized using GraphPad Prism 8.0.2 software ("GraphPad Software", USA) [24]. Signifcance ($p<0.05$) was determined by two-way ANOVA and Tukey's multiple comparison test for all data as described before [25].

Results and Discussion.

H^+/K^+ Fluxes in E. coli Wild Type, Hyd-1 and Hyd-2 Mutants When Cells Were Grown on Low Concentration (2 $g \cdot L^{-1}$) of Glucose. When the bacteria were grown at 2 $g \cdot L^{-1}$ glucose medium and during assays low and high glucose was supplemented total proton flux (J_{H^+}) at rates of 1.5 $mmol \cdot min^{-1}$ and 2.26 $mmol \cdot min^{-1}$ per 10^9 cells were observed, respectively (Fig. 1, a). In *hyaD* and *hybG* total J_{H^+} increased by 20%, only in *hybF* increased by 30% and in other mutants remained similar compared to wild type. When cells were treated with DCCD no significant changes were observed in Hyd-1 deletion mutants, meanwhile in *hybD, hybG, hybE* J_{H^+} increased by 20%, 35% and 17%, respectively. Interestingly most significant changes observed when considering DCCD-sensitive fluxes. Particularly, in *hyaD* and *hyaF* DCCD-sensitive J_{H^+} increased by 50%, in *hyaE* – by 20% (Fig. 1, b). It was shown previously that under aerobic conditions HyaE functions as a chaperone protein that is non-essential for the cell's survival or function but plays a role in assisting the β subunit (HyaA) of the hydrogenase isoenzyme 1 in *E. coli* [26].

Moreover, under aerobic conditions, a *hyaE* mutant does not show a serious defect in fumarate-dependent hydrogen oxidation, and Hyd-1 appears to be localized to the membrane correctly [11]. In this study, conducted under fermentative conditions with low glucose levels, it was found that HyaE is not critical for the activity of Hyd-1 or the maturation of HyaA. This conclusion is based on the significant differences observed in the behavior of a *hyaA* deletion mutant, which notably affects proton fluxes, compared to the effects seen with *hyaE* (data not shown).

HyaD is similar to HybD, which is required for the maturation for Hyd-2 [9, 27]. It is therefore likely that HyaD is an endopeptidase required for maturation of Hyd-1. Thus, above-mentioned DCCD-sensitive J_{H^+} increase in *hyaD* are in good confirmation with data observed in *hyaB* deletion mutant (data not shown), where again significant increase in DCCD-sensitive J_{H^+} was observed, suggesting that under implemented conditions *hyaD* and *hyaF* genes are important for Hyd-1 activity and specifically for the functioning of HyaB catalytic subunit. Totally, different DCCD-sensitivity was observed in *hyb* deletion mutants, in *hybD* DCCD-sensitive J_{H^+} decreased by ~30%, in *hybG* and *hybE* decreased by ~50%, meanwhile in *hybF* it increased by ~60% (Fig. 1, b).

Data show that HybD an endopeptidase involved in the maturation of the HybC [6], particularly is required for assembly of the catalytically active HybC-HybO heterodimer [28] is not as essential as *hybG* and *hybE*, where the contribution of F_0F_1-ATPase was stronglysuppressed. Data for *hybE* and *hybF* were similar to *hybC* (data not shown) strongly suggesting that in these conditions this two proteins are important for the Hyd-2 activity. Moreover, this data are in good confirmation with previous research of Sargent's group showing that *hybE* deletion resulted in a very low hydrogenase 2 activity [29]. Interestingly only in *hybF* DCCD-sensitive flux increased, which states the cross-regulation between hydrogenases,

particularly the role of *hybF* for the activity of Hyd-1 as obtained difference had more similarity with *hyaB* data. *HybF* has homology with *hypA*, and it was shown that during growth in TGYEP medium in a presence of 0.8% glycerol it is involved in the maturation of Hyd-1 and Hyd-2 [30]. Thus, in a presence of 0.2% glucose most possible it is not involved in a maturation of Hyd-2. The introduction of high glucose concentrations significantly affects the J_{H^+} transport in specific mutants, particularly observed in *hyaD* and *hyaF*, where there is a notable decrease of approximately 30% in total J_{H^+} compared to the wild type, which, indeed differ from data obtained during addition of low glucose.

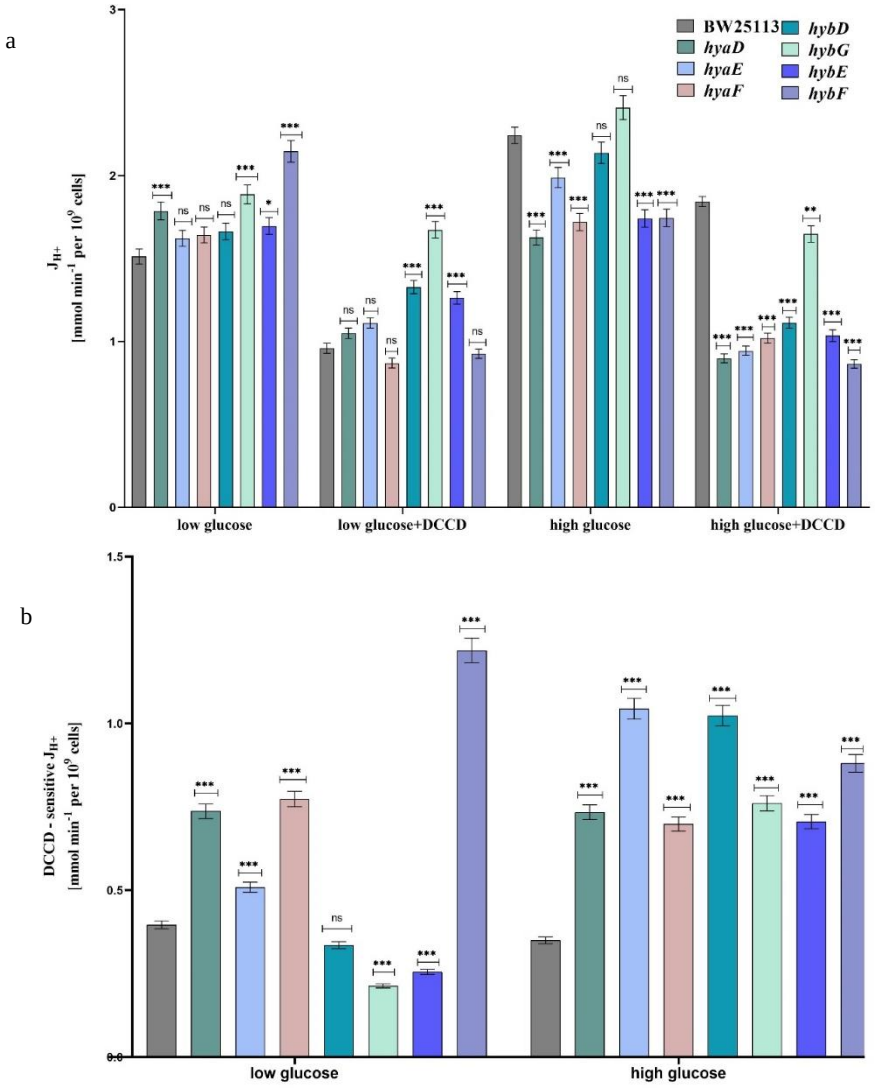

Fig. 1. Total (a) and DCCD-sensitive (b) J_{H^+} by whole cells of *E. coli* BW25113 wild type, single deletion *hya*, and *hyb* mutant strains under fermentative conditions at pH 7.5 and 37°C in the presence of 2 $g \cdot L^{-1}$ glucose. The assay pH (7.5) matched the growth pH. During the assay, either 2 $g \cdot L^{-1}$ or 8 $g \cdot L^{-1}$ glucose was supplemented. DCCD (0.2 *mM*) was added to the assay medium to selectively inhibit F_0F_1–ATPase activity when indicated.

Interestingly, despite the essential roles of *hyaD* and *hybD* as specialized proteases in the processing of hydrogenase subunits and the similar crucial functions of *hybF* and *hybG* in nickel processing and chaperoning, respectively, *hybD* and *hybG* subunits exhibited no significant differences in J_{H^+} transport under high glucose conditions. This resilience contrasts with the decrease observed in *hyaD*, *hyaF*, *hybE*, and *hybF*, suggesting that the roles these proteins play in hydrogenase assembly and maturation do not uniformly influence their sensitivity to glucose concentration changes.

The observed increase in DCCD-sensitive proton flux in *hybG* and *hybE* by approximately 50%, and even more so in *hybD* and *hybF* by 60% might reflect a compensatory mechanism aimed at maintaining energy transduction efficiency under conditions that otherwise impair specific hydrogenase activities. Definitely, these proteins have impact on both Hyd-1 and Hyd-2 membrane subunits, as in all of them similar to membrane-subunit absent mutants when grown in a presence of low glucose concentration and supplemented with high glucose DCCD-sensitive fluxes and contribution of F_0F_1–ATPase were strongly increased (data not shown). Given the identified and hypothesized roles of these gene products in cofactor processing and chaperoning, the data suggest a regulatory mechanism that balances hydrogenase assembly and function with the metabolic state of the cell, as indicated by glucose availability.

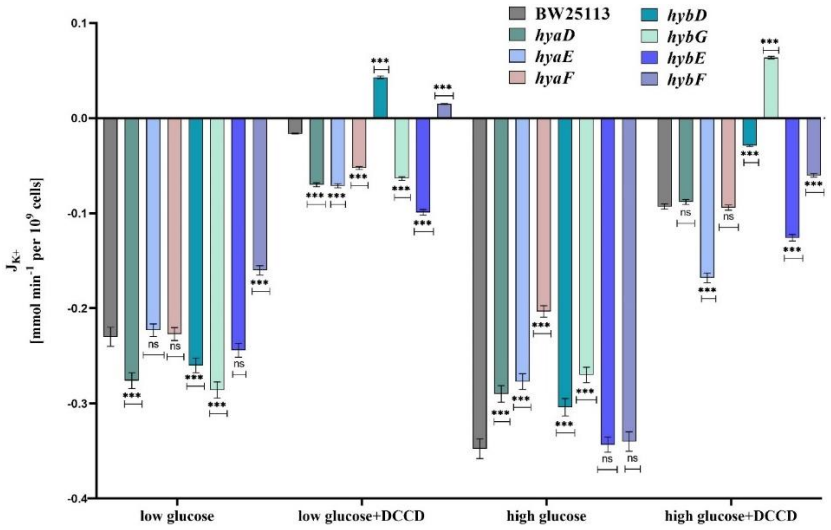

Fig. 2. Total J_{K^+} by whole cells of *E. coli* BW25113 wild type, single deletion *hya*, and *hyb* mutant strains under fermentative conditions at pH 7.5 and 37°C in the presence of 2 $g \cdot L^{-1}$ glucose. The assay pH (7.5) matched the growth pH. During the assay, either 2 $g \cdot L^{-1}$ or 8 $g \cdot L^{-1}$ glucose was supplemented. DCCD (0.2 *mM*) was added to the assay medium to selectively inhibit F_0F_1–ATPase activity when indicated. A positive value indicates efflux and a negative value indicates influx.

Total J_{K^+} were 0.23 $mmol \cdot min^{-1}$ and 0.35 $mmol \cdot min^{-1}$ when during assays separately low and high glucose was supplemented (Fig. 2). Upon adding low glucose, *hyaE* and *hyaF* mutants did not show notable changes. However, a 20% increase in J_{K^+} was observed in the *hyaD* mutant, a finding paralleled in the *hyaC* mutant under the same conditions. This increase in the *hyaC* mutant was significantly

reliant on F_0F_1–ATPase activity. Unlike *hyaD*, no variation in DCCD-sensitive J_{K^+} compared to the wild type was detected. Conversely, a reduction of approximately 25% in DCCD-sensitive J_{K^+} was noted in both *hyaE* and *hyaF* mutants, aligning with findings in *hyaB* mutant (unreported data). In *hyb* deletion mutants, a notable reduction of about 30% in total J_{K^+} was seen, particularly in *hybF*. Siginificant differences were observed in DCCD-sensitive fluxes, namely in *hybD* DCCD-sensitive J_{K^+} increased by ~30%, while in *hybE* and *hybF* it decreased by 30%. Moreover, observation for *hybF* was conditioned by F_0F_1–ATPase.

Data differ when high glucose was supplemented: in *hyaD* total and DCCD-sensitive J_{K^+} decreased by ~20%, simply suggesting that absence of this protein affects the flux conditioned by F_0F_1–ATPase. In *hyaE* and *hyaF* total J_{K^+} decreased by 20% and 40%, respectively, meanwhile, DCCD-sensitive fluxes decreased by 60%. Interestingly from *hyb* deletion only total J_{K^+} was decreased by 25%, meanwhile DCCD-sensitive flux increased to the same extent.

H^+/K^+ Fluxes in E. coli Wild Type, Hyd-1 and Hyd-2 Mutants when Cells were Grown on Low Concentration (8 $g \cdot L^{-1}$) of Glucose. When wild type cells were grown at 8 $g \cdot L^{-1}$ glucose medium and during assays low and high glucose was supplemented total proton flux (J_{H^+}) was 1.95 $mmol \cdot min^{-1}$ per 10^9 cells (Fig. 3, a). When low glucose was supplemented during assays in all tested mutants minor changes up to 15% were observed in total J_{H^+}, and only in *hyaF* it was decreased by 25%. When high glucose was supplemented again minor differences were observed, excepting *hyaD* with 27% increase in total J_{H^+}.

Under low glucose conditions, the mutants demonstrated varied DCCD-sensitivity to the decreased availability of glucose. The *hyaD* mutant showed a substantial decrease in DCCD-sensitive J_{H^+}, with a 65% reduction compared to the wild type (Fig. 3, b). Similarly, *hyaE* experienced a 30% reduction in DCCD-sensitive J_{H^+}. HyaD and HyaE proteins appear to be involved in the processing of Hyd-1 structural subunits, HyaB and HyaA, respectively, potentially acting together as a processing complex. This suggests that HyaD and HyaE are crucial for preparing the Hyd-1 enzyme's structural components for proper assembly and function [27]. Given significant decreases underscores its either the critical dependency on glucose for proper hydrogenase maturation or multi-component cross-regulation is operating specifically when cells were grown in a presence of high glucose, which still need a further research.

In *hyaF*, proton flux decreased by 70%. This severe reduction highlights HyaF susceptibility to glucose levels, likely due to its integral role in nickel integration and activation of the Hyd-1 enzyme [27].

In contrast, the *hyb* mutants presented a different pattern of response. When cells were grown in presence of high glucose concentration and during assays low glucose was supplemented in all *hyb* mutants total J_{H^+} did not significantly change. In *hybD* DCCD-sensitive J_{H^+} remained unchanged, indicating its function in the maturation of the HybC subunit, as both in low glucose and high glucose assays data for both *hybD* and *hybC* were similar (data not shown). *hybE* also showed no significant change, aligning with *hybD*, which may reflect a similar resilience or an

alternative pathway ensuring its participation in hydrogenase activity remains unaffected. However, *hybF* exhibited an increase in DCCD-sensitive J_{H^+} by 38%, unique among the mutants. *HybF* is believed to fulfil a role similar to that of HypA, focusing on nickel processing within the hydrogenase enzymes [30, 31]. Thus, *hybF* in depending on applied glucose concentration is most probably is responsible for Hyd-1, rather than for Hyd-2, as in all discussed conditions data from *hybF* were more similar to *hya* membrane-subunit deletion mutants (data not shown).

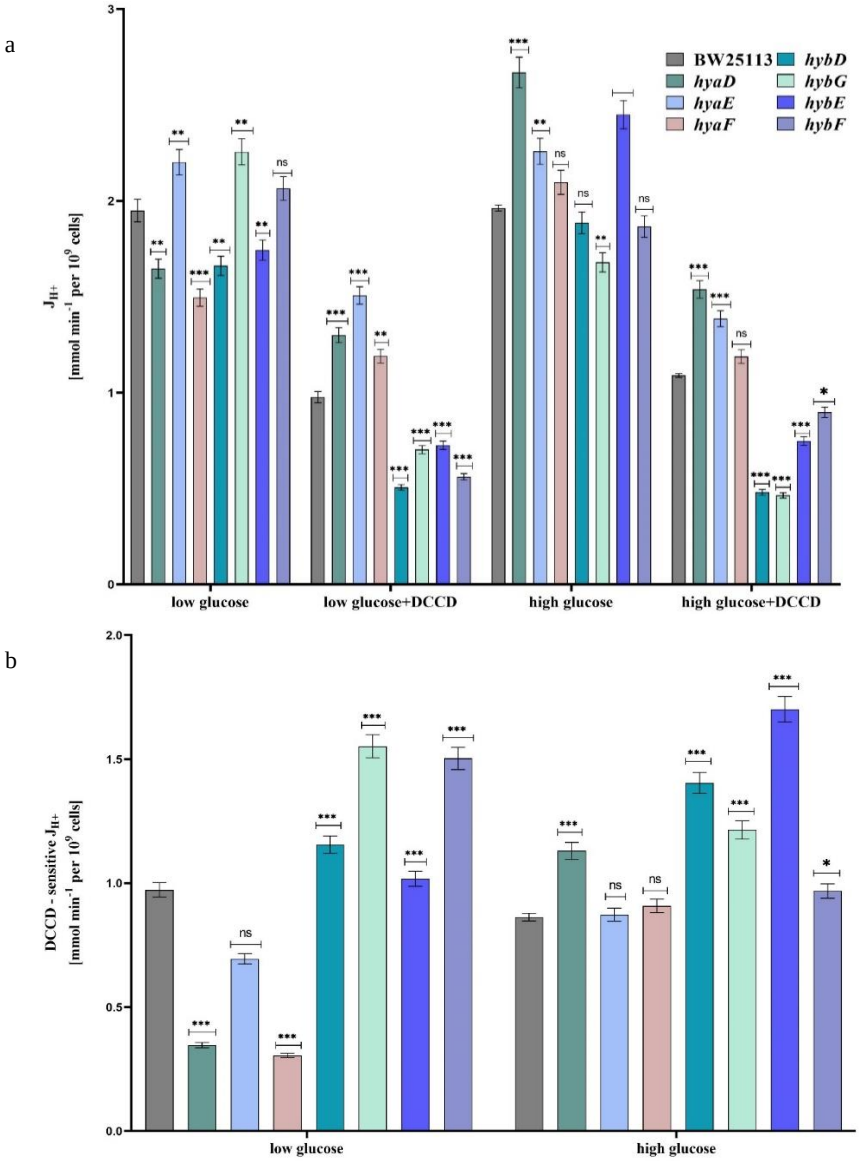

Fig. 3. Total (a) and DCCD-sensitive (b) J_{H^+} by whole cells of *E. coli* BW25113 wild type, single deletion *hya*, and *hyb* mutant strains under fermentative conditions at pH 7.5 and 37°C in the presence of 8 $g \cdot L^{-1}$ glucose. The assay pH (7.5) matched the growth pH. During the assay, either 2 $g \cdot L^{-1}$ or 8 $g \cdot L^{-1}$ glucose was supplemented. DCCD (0.2 *mM*) was added to the assay medium to selectively inhibit F_0F_1–ATPase activity when indicated.

When high glucose was applied in *hyaD*, with a 24% increase in total and DCCD-sensitive J_{H^+}, contrasts its response under low glucose, suggesting a complex glucose-sensing mechanism that modulates its proteolytic function. *HybD* displayed a notable 40% increase in DCCD-sensitive J_{H^+}, indicating that glucose might activate or enhance its role in assembling the active HybC-HybO heterodimer. In *hybE* DCCD-sensitive J_{H^+} increase by 50% under high glucose conditions implies that elevated glucose concentrations could enhance its stability or activity, optimizing Hyd-2 assembly in nutrient-rich environments. Interestingly, *hybF* did not show significant change, suggesting a threshold effect where low glucose activates the mutant, but high glucose does not further influence it (Fig. 3, b).

The alterations in potassium uptake observed in *E. coli* mutants, each lacking different maturational subunits for the hydrogenases Hyd-1 and Hyd-2, reveal a tightly regulated metabolic adaptation to varying glucose concentrations, a reflection of their roles beyond mere hydrogenase maturation. When cells were grown in the presence of high glucose concentration and during assays low and high glucose was supplemented total J_{K^+} was 0.2 $mmol \cdot min^{-1}$ per 10^9 cells (Fig. 4).

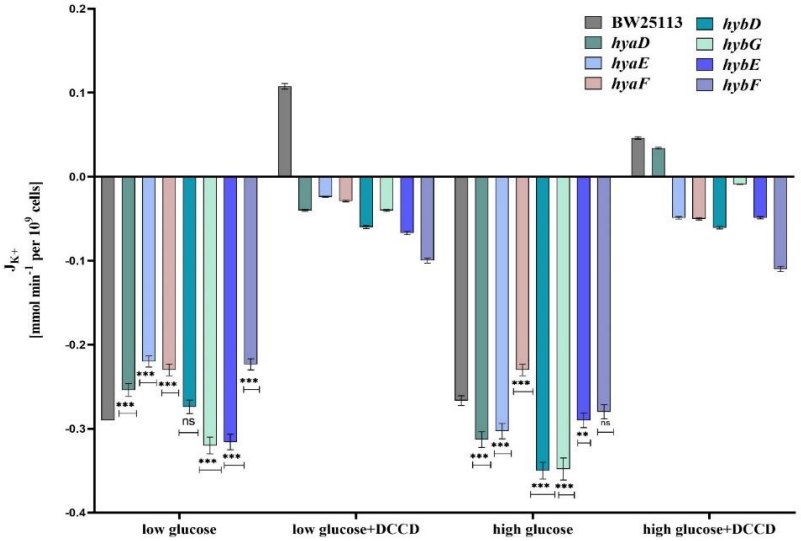

Fig. 4. Total J_{K^+} by whole cells of *E. coli* BW25113 wild type, single deletion *hya* and *hyb* mutant strains under fermentative conditions at pH 7.5 and 37°C in the presence of 8 $g \cdot L^{-1}$ glucose. The assay pH (7.5) matched the growth pH. During the assay, either 2 $g \cdot L^{-1}$ or 8 $g \cdot L^{-1}$ glucose was supplemented. DCCD (0.2 *mM*) was added to the assay medium to selectively inhibit F_0F_1–ATPase activity when indicated. A positive value indicates efflux and a negative value indicates influx.

Under low glucose conditions, the trend across most mutants is an increase in potassium uptake. *HyaD*, with an 14% decrease, *hyaE* and *hyaF*, with a 23% decrease, and the *hyb* subunits, with no significant differences, except *hybF* with 25% decrease, suggest an upregulation of potassium uptake as a compensatory mechanism during energy limitation. It is worth mentioning when similar experiments were conducted with membrane-bound subunits; no significant differences in total fluxes were observed. The potassium flux was totally F_0F_1-dependent. The slight decrease in total J_{K^+} shown in *hyaE*, however, indicates that not all maturational subunits react to low glucose by increasing potassium uptake, which could point to specific roles that these

proteins play in the cell's metabolism, which might be less affected by energy limitations or, perhaps, compensated by other metabolic pathways.

When exposed to high glucose concentrations, all mutants show an increase in J_{K^+}: the minor increases in J_{K^+} for *hyaD* (23%), *hyaE* (34%), and particularly the *hyb* mutants, with *hybD* and *hybG* both at 25%. Meanwhile in *hybE* and *hybF* no significant changes were observed. This and previously obtained data suggest that when high glucose is applied the role of Hyd-2 and consequently the role of maturational subunits do not have a primary role at least in proton-potassium transport. High glucose availability could enhance processes that demand higher potassium levels for optimal function, such as enzyme activation, osmoregulation, or ion balance to cope with the increased metabolic activity.

These observations underline the intricate balance between glucose availability, hydrogenase maturation, and bacterial energy metabolism. The distinct responses of the *hya* and *hyb* mutants to glucose conditions reflect their specialized roles in hydrogenase enzyme maturation and activity, emphasizing the complex regulatory mechanisms that adapt bacterial energetics to environmental changes. There are additional, yet unidentified, factors critical for converting the hydrogenase precursors into their active form, or mechanisms that regulate the enzyme's activity after it's produced.

Conclusion. The results reveal the crucial roles of specific maturation proteins in influencing hydrogenase activity, showing that mutations in these proteins significantly affect ion fluxes in a glucose presence. Specifically, alterations in *hyaD* and *hyaF* led to a notable increase in proton flux, underlining their essential roles in Hyd-1 activity. The study also suggests a cross-regulation between hydrogenases, with the *hybF* mutant displaying changes in proton flux indicative of its involvement in the maturation or activity of both Hyd-1 and Hyd-2 under certain conditions.

An important finding is the observation that in the *hyaF* mutant, J_{H^+} was decreased by 70% suggesting that HyaF function is significantly influenced by the glucose concentration.

Furthermore, the study underscores the influence of glucose concentration on *E. coli* metabolic pathways, with marked effects on the functionality of hydrogenase enzymes and associated ion fluxes. Gene products that process cofactors and assist in protein folding for hydrogenase enzymes seem to affect the assembly and function of these enzymes, varying with glucose availability.

In conclusion, the research enhances our comprehension of the intricate interrelations between hydrogenase maturation. It highlights the complex regulatory mechanisms that allow bacteria to adjust their energy production strategies according to environmental changes. These insights could inform biotechnological applications aiming to optimize energy generation through microbial processes, considering the critical role of specific maturational subunits in enzyme activity and the overarching influence of glucose on microbial energetics.

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REFERENCES

1. Trchounian K., Poladyan A., et al. Multiple and Reversible Hydrogenases for Hydrogen Production by *Escherichia coli* : Dependence on Fermentation Substrate, pH and the F_0F_1–TPase. *Crit. Rev. Biochem. Mol. Biol.* **47** (2012), 236–249. https://doi.org/10.3109/10409238.2012.655375
2. Forzi L., Sawers R.G. Maturation of [NiFe]-Hydrogenases in *Escherichia coli*. *BioMetals* **20** (2007), 565. https://doi.org/10.1007/s10534-006-9048-5
3. Böck A., King P.W., et al. Maturation of Hydrogenases. *Advances in Microbial Physiology* **51** (2006), 1–71, 225. https://doi.org/10.1016/S0065-2911(06)51001-X
4. Pinske C., Jaroschinsky M., et al. Zymographic Differentiation of [NiFe]-Hydrogenases 1, 2 and 3 of *Escherichia coli* K-12. *BMC Microbiol*. **12** (2012), 134. https://doi.org/10.1186/1471-2180-12-134
5. Pinske C., McDowall J.S., et al. Analysis of Hydrogenase 1 Levels Reveals an Intimate Link between Carbon and Hydrogen Metabolism in *Escherichia coli* K-12. *Microbiology (NY)* **158** (2012), 856–868. https://doi.org/10.1099/mic.0.056622-0
6. Soboh B., Krüger S., et al. Development of a Cell-free System Reveals an Oxygen-Labile Step in the Maturation of [NiFe]-Hydrogenase 2 of *Escherichia coli*. *FEBS Lett.* **584** (2010), 4109–4114. https://doi.org/10.1016/j.febslet.2010.08.037
7. Pinske C., Jaroschinsky M., et al. Physiology and Bioenergetics of [NiFe]-Hydrogenase 2-Catalyzed H 2-Consuming and H 2-Producing Reactions in *Escherichia coli*. *J. Bacteriol*. **197** (2015), 296–306. https://doi.org/10.1128/JB.02335-14
8. Lacasse M.J., Zamble D.B. [NiFe]-Hydrogenase Maturation. *Biochemistry* **55** (2016), 1689–1701. https://doi.org/10.1021/acs.biochem.5b01328
9. Fritsche E., Paschos A., et al. Crystal Structure of the Hydrogenase Maturating Endopeptidase HYBD from *Escherichia coli*. *J. Mol. Biol.* **288** (1999), 989–998. https://doi.org/10.1006/jmbi.1999.2719
10. Theodoratou E., Huber R., Böck A. [NiFe]-Hydrogenase Maturation Endopeptidase: Structure and Function. *Biochem. Soc. Trans.* **33** (2005), 108–111. https://doi.org/10.1042/BST0330108
11. Dubini A. How Bacteria Get Energy from Hydrogen: a Genetic Analysis of Periplasmic Hydrogen Oxidation in *Escherichia coli*. *Int. J. Hydrogen. Energy* **27** (2002), 1413–1420. https://doi.org/10.1016/S0360-3199(02)00112-X
12. Palmer T., Sargent F., Berks B.C. Export of Complex Cofactor-Containing Proteins by the Bacterial Tat Pathway. *Trends Microbiol*. **13** (2005), 175–180. https://doi.org/10.1016/j.tim.2005.02.002
13. August Bock G.S. *Fermentation* (2006).
14. Clark D. The Fermentation Pathways of *Escherichia coli*. *FEMS Microbiol. Rev.* **63** (1989), 223–234. https://doi.org/10.1016/0168-6445(89)90033-8
15. Lindenstrauß U., Skorupa P., et al. The Dual-Function Chaperone HycH Improves Assembly of the Formate Hydrogenlyase Complex. *Biochemical Journal* **474** (2017), 2937–2950. https://doi.org/10.1042/BCJ20170431
16. Trchounian K., Trchounian A. Hydrogen Producing Activity by *Escherichia coli* Hydrogenase 4 (*hyf*) Depends on Glucose Concentration. *Int. J. Hydrogen. Energy* **39** (2014), 16914–16918. https://doi.org/10.1016/j.ijhydene.2014.08.059
17. Vanyan L., Trchounian K., *HyfF* Subunit of Hydrogenase 4 is Crucial for Regulating F_0F_1-dependent Proton/Potassium Fluxes during Fermentation of Various Concentrations of Glucose. *J. Bioenerg. Biomembr.* **54** (2022), 69–79. https://doi.org/10.1007/s10863-022-09930-x
18. Mikoyan G., Karapetyan L., et al. External Succinate and Potassium Ions Influence Dcu Dependent F_0F_1–ATPase Activity and H^+ Flux of *Escherichia coli* at Different pHs. *J. Bioenerg. Biomembr.* **52** (2020), 377–382. https://doi.org/10.1007/s10863-020-09847-3

19. Trchounian K., Trchounian A., Hydrogenase 2 is Most and Hydrogenase 1 is Less Responsible for H_2 Production by *Escherichia coli* under Glycerol Fermentation at Neutral and Slightly Alkaline pH. *Int. J. Hydrogen. Energy* **34** (2009), 8839–8845. https://doi.org/10.1016/j.ijhydene.2009.08.056
20. Baba T., Ara T., et al. Construction of *Escherichia coli* K-12 in-Frame, Single-Gene Knockout Mutants: the Keio Collection. *Mol. Syst. Biol.* **2** (2006), 0008. https://doi.org/10.1038/msb4100050
21. Rodriguez-Navarro A., Blatt M.R., Slayman C.L. A Potassium-Proton Symport in *Neurospora crassa*. *J. Gen. Physiol.* **87** (1986), 649–674. https://doi.org/10.1085/jgp.87.5.649
22. Hakobyan B., Pinske C., et al. pH and a Mixed Carbon-Substrate Spectrum Influence FocA- and FocB-dependent, Formate-Driven H_2 Production in *Escherichia coli*. *FEMS Microbiol. Lett.* **365** (2018). https://doi.org/10.1093/femsle/fny233
23. Karapetyan L., Mikoyan G., et al. *Escherichia coli* Dcu C4-Dicarboxylate Transporters Dependent Proton and Potassium Fluxes and F_0F_1–ATPase Activity during Glucose Fermentation at pH 7.5. *Bioelectrochemistry* **141** (2021), 107867. https://doi.org/10.1016/j.bioelechem.2021.107867
24. Lemus M.B., Bayliss J.A., et al. A Stereological Analysis of NPY, POMC, Orexin, GFAP Astrocyte, and Iba1 Microglia Cell Number and Volume in Diet-Induced Obese Male Mice. *Endocrinology* **156** (2015), 1701–1713. https://doi.org/10.1210/en.2014-1961
25. Gevorgyan H., Abaghyan T., et al. Propionic and Valproic Acids Have an Impact on Bacteria Viability, Proton Flux and ATPase Activity. *J. Bioenerg. Biomembr.* **55** (2023), 397–408. https://doi.org/10.1007/s10863-023-09983-6
26. Dubini A., Sargent F. Assembly of Tat-dependent [NiFe] Hydrogenases: Identification of Precursor-Binding Accessory Proteins. *FEBS Lett.* **549** (2003), 141–146. https://doi.org/10.1016/S0014-5793(03)00802-0
27. Menon N.K., Robbins J., et al. Mutational Analysis and Characterization of the *Escherichia coli hya* Operon, Which Encodes [NiFe] Hydrogenase 1. *J. Bacteriol.* **173** (1991), 4851–4861. https://doi.org/10.1128/jb.173.15.4851-4861.1991
28. Senger M., Stripp S.T., Soboh B. Proteolytic Cleavage Orchestrates Cofactor Insertion and Protein Assembly in [NiFe]-Hydrogenase Biosynthesis. *J. Biol. Chem.* **292** (2017), 11670–11681. https://doi.org/10.1074/jbc.M117.788125
29. Jack R.L., Buchanan G., et al. Coordinating Assembly and Export of Complex Bacterial Proteins. *EMBO J.* **23** (2004), 3962–3972. https://doi.org/10.1038/sj.emboj.7600409
30. Hube M., Blokesch M., Böck A. Network of Hydrogenase Maturation in *Escherichia coli* : Role of Accessory Proteins HypA and HybF. *J. Bacteriol.* **184** (2002), 3879–3885. https://doi.org/10.1128/JB.184.14.3879-3885.2002
31. Menon N.K., Chatelus C.Y., et al. Cloning, Sequencing, and Mutational Analysis of the *hyb* Operon Encoding *Escherichia coli* Hydrogenase 2. *J. Bacteriol.* **176** (1994), 4416–4423. https://doi.org/10.1128/jb.176.14.4416-4423.1994

Լ. Մ. ՎԱՆՅԱՆ

ՊՐՈՏՈՆԻ ԵՎ ԿԱԼԻՈՒՄԻ ՀՈՍՔԵՐԸ *ESCHERICHIA COLI*-Ի ՀԻԴ-1 ԵՎ ՀԻԴ-2-Ի ՀՈՒՍԱՆԱՑՄԱՆ ՀԱՄԱՐ ՊԱՏԱՍԽԱՆԱՏՈՒ ԵՆԹԱՄԻԱՎՈՐՆԵՐԻ ԽԱԽՏՈՒՄՆԵՐՈՎ ՄՈՒՏԱՆՏՆԵՐՈՒՄ ԳԼՅՈՒԿՈԶԻ ԽՄՈՐՄԱՆ ՊԱՅՄԱՆՆԵՐՈՒՄ

Հետազոտության նպատակն է ուսումնասիրել Հիդ-1-ի և Հիդ-2-ի հատուկ ենթամիավորների դերը և դրանց ազդեցությունը պրոտոնի և

կալիումի հոսքերի վրա *Escherichia coli*-ում գլյուկոզի խմորման ընթացքում: Ուսումնասիրելով գլյուկոզի տարբեր հասանելիության պայմանները՝ մենք փորձեցինք պարզել, թե ինչպես են այս ենթամիավորներն ազդում *E. coli*-ի նոյութափոխանակային հարմարվողականության վրա խմորման պայմաններում՝ փոխելով իոնային հոսքերը, որն էլ թույլ կտա բացահայտել դրանց դերը հիդրոգենազի ակտիվության և բջջին էներգիական նյութափոխանակության մեջ: Այս արդյունքները նպաստում են մանրէների ֆիզիոլոգիայի մեր ըմբռնմանը՝ ընդգծելով էներգետիկ նյութափոխանակության, իոնային հոմեոստազի և շրջակա միջավայրի պայմանների փոխազդեցությունը: Հատկանշական է, որ *hyaD* և *hyaF* մուտանտներում ԴՑԿԴ-զգայուն (N, N’-դիցիկլոհեքսիլկարբոդիիմիդ) պրոտոնային հոսքը ավելացե;լ է 50%-ով ընդգծելով նրանց կարևոր դերը Հիդ-1 ակտիվության մեջ: Բացառապես, *hybF* մուտանտում դիտվել է ԴՑԿԴ-զգայուն պրոտոնային հոսքի ավելացում, որը խիստ նման է եղել *hyaB*-ում դիտված տվյալներին՝ հստակ ցույց տալով հիդրոգենազների միջև փոխադարձ-խաչաձև կարգավորումը՝ հատկապես ընդգծելով HybF-ի դերը Հիդ-1-ի ակտիվության մեջ: Այսպիսով, ցածր գլյուկոզի առկայության դեպքում HybF-ն ենթադրաբար ավելի քիչ է մասնակցում Հիդ-2-ի հասունացմանը: *hybE*-ի և *hybF*-ի մուտանտների տվյալները հստակ ցույց են տալիս, որ վերջիններս խիստ կարևոր են Հիդ-2-ի հասունացման համար երբ մանրէներն աճեցվել են քիչ գլյուկոզի առկայությամբ, իսկ փորձերի ընթացքում ավելացվել է գլյուկոզի բարձր կոնցենտրացիա: *hyaD* և *hyaF* մուտանտներում դիտվել է ԴՑԿԴ զգայուն պրոտոնային հոսքի 50% աճ, ինչը ցույց է տալիս նրանց կարևոր դերը Հիդ-1 գործունեության մեջ: Այսպիսով, ստացված արդյունքները ցույց են տալիս, որ կախված գլյուկոզի կոնցենտրացիայից՝ հասունացմանը մասնակցող հատուկ ենթամիավորները կարող են փոփոխված և խաչաձև կարգավորվող դերեր ունենալ Հիդ ֆերմենտների հասունացման գործում:

Л. М. ВАНЯН

ПОТОКИ ПРОТОНОВ И КАЛИЯ У МУТАНТОВ *ESCHERICHIA COLI* С ДЕФЕКТАМИ СУБЪЕДИНЦ, ОТВЕТСТВЕННЫХ ЗА СОЗРЕВАНИЕ ГИД-1 И ГИД-2 ПРИ БРОЖЕНИИ ГЛЮКОЗЫ

В этом исследовании изучается роль конкретных субъединиц Гид-1 и Гид-2 и их влияние на потоки протонов и калия при брожении глюкозы в *Escherichia coli* (*E. coli*). Исследуя условия различной доступности глюкозы, мы стремились выяснить, как эти субъединицы влияют на метаболическую адаптацию *E. coli* в условиях брожения путем изменения потоков ионов, что указывает на их роль в активности гидрогеназы и клеточном энергетическом метаболизме. Примечательно, что мутанты *hyaD* и *hyaF* продемонстрировали 50% увеличение ДЦКД (N,N'-дициклогексилкарбодиимид)-чувствительного потока протонов, что подчеркивает их решающую роль в активности Гид-1. Мутант *hybF* продемонстрировал значительное увеличение DCCD-чувствительного потока, что напоминает данные по наблюдениям за *hyaB*, это

указывает на перекрестную регуляцию между гидрогеназами и подчеркивает роль HybF в активности Гид-1. Таким образом, в присутствии низкого уровня глюкозы HybF, по-видимому, меньше участвует в созревании Гид-2. Данные для *hybE* и *hybF* убедительно свидетельствуют о том, что в условиях роста при низкой концентрации глюкозы дополнение глюкозы высокой концентрации имеет решающее значение для активности Гид-2. Мутанты *hyaD* и *hyaF* продемонстрировали увеличение потока протонов, чувствительного к ДЦКД, на 50%, что указывает на их важную роль в активности Гид-1. В совокупности наши результаты показывают, что в зависимости от концентрации глюкозы определенные субъединицы могут иметь измененную и перекрестно-регулируемую роль в созревании ферментов Гид.