Biochimica et Biophysica Acta 1787 (2009) 691–696

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

# The ins and outs of $Na^+$ bioenergetics in *Acetobacterium woodii*

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ARTICLE INFO

*Article history:*
Received 3 December 2008
Received in revised form 30 December 2008
Accepted 30 December 2008
Available online 8 January 2009

*Keywords:*
Acetogen
*Acetobacterium woodii*
Energy conservation
Caffeate respiration
Rnf
ATP synthase

ABSTRACT

The acetogenic bacterium *Acetobacterium woodii* uses a transmembrane electrochemical sodium ion potential for bioenergetic reactions. A primary sodium ion potential is established during carbonate (acetogenesis) as well as caffeate respiration. The electrogenic $Na^+$ pump connected to the Wood–Ljungdahl pathway (acetogenesis) still remains to be identified. The pathway of caffeate reduction with hydrogen as electron donor was investigated and the only membrane-bound activity was found to be a ferredoxin-dependent $NAD^+$ reduction. This exergonic electron transfer reaction may be catalyzed by the membrane-bound Rnf complex that was discovered recently and is suggested to couple exergonic electron transfer from ferredoxin to $NAD^+$ to the vectorial transport of $Na^+$ across the cytoplasmic membrane. Rnf may also be involved in acetogenesis. The electrochemical sodium ion potential thus generated is used to drive endergonic reactions such as flagellar rotation and ATP synthesis. The ATP synthase is a member of the $F_1F_O$ class of enzymes but has an unusual and exceptional feature. Its membrane-embedded rotor is a hybrid made of $F_O$ and $V_O$-like subunits in a stoichiometry of 9:1. This stoichiometry is apparently not variable with the growth conditions. The structure and function of the Rnf complex and the $Na^+$ $F_1F_O$ ATP synthase as key elements of the $Na^+$ cycle in *A. woodii* are discussed.



## 1. Introduction

Acetogenic bacteria are a specialized group of strictly anaerobic bacteria that are ubiquitous in nature and found in nearly every ecosystem, even at high temperatures, acidic pH values or saline systems [1]. Together with the methane forming archaea they constitute the last limbs in the anaerobic food web that ultimately leads to the production of methane from polymers in the absence of oxygen. Acetogens are defined as organisms able to reduce $CO_2$ to acetate *via* the acetyl-CoA pathway, also termed the Wood–Ljungdahl pathway or CO-dehydrogenase pathway (Fig. 1) [2,3]. This metabolic capability differentiates acetogens from organisms that synthesize acetate by other metabolic pathways. However, phylogenetically acetogens are rather diverse. They are exclusively found in the domain *Bacteria*. Currently, there are 21 bacterial genera that contain approximately 100 reported acetogenic species [1]. In some genera, e.g. *Acetobacterium* and *Sporomusa*, the members are exclusively acetogenic. However, many acetogens can be found in genera containing both acetogenic and non-acetogenic bacteria (e.g., *Clostridium*, *Ruminococcus*, *Eubacterium*, *Thermoanaerobacter*, *Treponema*).

Acetogenic bacteria or acetogens are among the metabolically most versatile anaerobic organisms. Most of them are able to grow chemoorganoheterotrophically on a variety of different organic substrates, including sugars or other $C_1$ compounds such as methoxylated aromatic compounds, dicarboxylic acids and alcohols [1]. In addition, they can grow autotrophically on $H_2 + CO_2$ [3]. Central to all these metabolic routes is the Wood–Ljungdahl pathway that was discovered while analyzing glucose fermentation in acetogens [4]. The hexose is fermented by way of glycolysis to pyruvate which is then oxidized by pyruvate:ferredoxin-oxidoreductase to acetyl-CoA, reduced ferredoxin and $CO_2$. The acetyl-CoA is then converted to acetate *via* acetyl phosphate. The oxidative branch of the pathway (Eq. (1)) is coupled to the synthesis of 4 mol of ATP by substrate level phosphorylation (SLP):

$$C_6H_{12}O_6 + 4ADP + 4Pi \rightarrow 2CH_3COOH + 2CO_2 + 4ATP + 4[H] \quad (1)$$

One of the interesting observations was that glucose was completely oxidized to three mol of acetate according to:

$$1C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad (2)$$

and, therefore, a pathway had to be postulated in which the two mol of $CO_2$ (produced in Eq. (1)) are reduced to acetate. By the pioneering work of Harland G. Wood and Lars G. Ljungdahl [5,6] the pathway was elucidated (Fig. 1) and it turned out that this pathway is not restricted to acetogens but widely distributed in the anaerobic world to fix carbon dioxide.

In the Wood–Ljungdahl pathway the reducing equivalents gained during glycolysis and pyruvate:ferredoxin-oxidoreductase are

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doi:10.1016/j.bbabio.2008.12.015

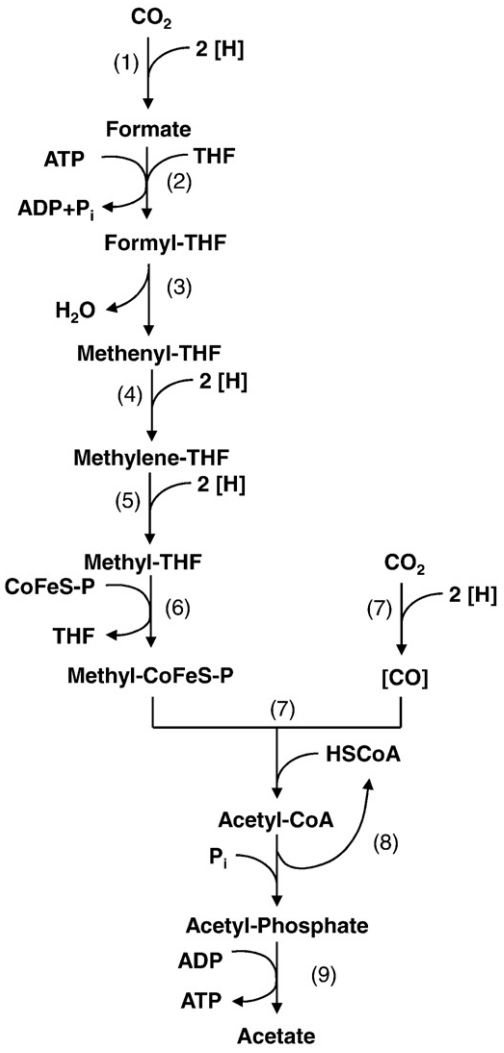


**Fig. 1.** The Wood–Ljungdahl pathway. Reductants are derived from oxidation of hydrogen or organic substrates. (1) formate dehydrogenase; (2) formyl-THF synthetase; (3) methenyl-THF cyclohydrolase; (4) methenyl-THF dehydrogenase; (5) methylene-THF reductase; (6) methyltransferase; (7) CO dehydrogenase/acetyl-CoA synthase; (8) phosphotransacetylase; (9) acetatekinase. [H] = reducing equivalent; THF = tetrahydrofolate; CoFeS-P = corrinoid iron sulfur protein; [CO] = enzyme-bound CO.

reoxidized by reducing the two mol of $CO_2$ to another mol of acetate according to:

$$2CO_2 + 4[H] + nADP + nP_i \rightarrow CH_3COOH + nATP \quad (3)$$

First, $CO_2$ is reduced to formate by action of formate dehydrogenase, then formate is activated and bound to tetrahydrofolate (THF), giving rise to formyl-THF (Fig. 1). Water is split off, and the resulting methenyl group is reduced *via* methylene-THF to methyl-THF. The methyl group is then transferred to a protein containing a corrinoid cofactor and iron sulfur clusters, the so called Co/FeS protein (Co/FeS-P). From there, the methyl group is transferred to the enzyme acetyl-CoA synthase (also called carbon monoxide dehydrogenase or CO-DH) and condensed on the enzyme with carbon monoxide, derived from another mol of $CO_2$ oxidized by the CO-DH activity of the acetyl CoA synthase, to acetyl-CoA [5,7,8]. Acetate is produced by action of phosphotransacetylase and acetate kinase. During growth on fermentable sugars, the Wood–Ljungdahl pathway serves as a sink for electrons, thereby allowing the organisms to produce 4 mol of ATP/mol hexose by substrate level phosphorylation; this is one of the highest ATP yields encountered by fermentative bacteria [9].

The Wood–Ljungdahl pathway also enables growth of acetogens on $H_2 + CO_2$ according to:

$$2CO_2 + 4H_2 + nADP + nP_i \rightarrow CH_3COOH + 2H_2O + nATP \quad (4)$$

and, therefore, it must be coupled to net ATP synthesis.

The overall free energy change ($\Delta G^{0\prime}$) in the Wood–Ljungdahl pathway is −95 kJ/mol and could allow for the synthesis of 1–2 mol of ATP. One mol of ATP is produced by SLP in the acetate kinase reaction, but one mol of ATP is consumed in the formyl-THF synthetase reaction (Fig. 1). Therefore, the net ATP gain by SLP is zero and ion gradient-driven phosphorylation must occur as well (because the organisms grow chemolithoautotrophically on $H_2 + CO_2$ according to Eq. 4). From a bioenergetic point of view, acetogens can be divided into two groups, the $Na^+$-dependent ones with *A. woodii* [10] and the $H^+$-dependent ones with *Moorella thermoacetica* (formerly *Clostridium thermoaceticum*) as model organisms [6]. The latter group contains cytochromes and a membrane-bound, $H^+$-motive electron transport chain as well as a $\Delta\mu H^+$-coupled $F_1F_O$ ATP synthase [11,12]. The $Na^+$-dependent acetogens lack cytochromes but have membrane-bound corrinoids [13]. Furthermore, experiments with *A. woodii* revealed the coupling of the Wood–Ljungdahl pathway to primary and electrogenic translocation of $Na^+$ across the cytoplasmic membrane [14]. The ion gradient established is used by a $Na^+$-translocating $F_1F_O$ ATP synthase for ATP synthesis [1,14]. The way the ion gradients ($H^+$ or $Na^+$) are established in acetogens are still an enigma but recent experiments that will be summarized here shed some light on the primary sodium ion pump that generates the electrochemical sodium ion potential in *A. woodii*. Furthermore, we will review some novel and unexpected features of the $\Delta\mu Na^+$ utilizer, the $F_1F_O$ ATP synthase of *A. woodii*.

## 2. Generation of a sodium motive force in *A. woodii*

Since acetogenesis from $H_2 + CO_2$ is coupled to the generation of a transmembrane electrochemical $Na^+$ potential, one of the enzymes of the Wood–Ljungdahl pathway must be membrane-bound and translocate $Na^+$ into the exterior of the cell. In analogy to methanogens that have a $Na^+$-motive membrane-bound, corrinoid-containing methyl-tetrahydromethanopterin:coenzmye M methyltransferase [15,16] it was thought that acetogens have a similar enzyme. This idea was fueled by the finding of membrane-bound corrinoids in *A. woodii* [13]. However, neither the methyltransferase nor another enzyme of the central carbon flow pathway could be localized at the cytoplasmic membrane. The enzyme was searched for two decades, but so far without success. The history of this search has been reviewed recently and the reader is referred to the literature [14]. Very recently, a possible solution to this enigma came from studies that addressed the biochemistry and bioenergetics of caffeate respiration in *A. woodii* that are described below.

### 2.1. Caffeate respiration in A. woodii

In addition to $CO_2$ alternative electron acceptors are used by *A. woodii*. It is known to reduce the carbon–carbon double bond of phenylacrylates such as caffeate as shown in Fig. 2 [17,18]. The electrons for caffeate reduction can be derived from various donors such as, for example, fructose, methanol or hydrogen [19]. It is important to note that *A. woodii* cannot use caffeate as carbon or as energy source but only reduces the double bond of caffeate yielding hydrocaffeate. Hydrocaffeate also is neither a carbon nor an energy source for *A. woodii*. Caffeate reduction is coupled to ATP synthesis by a chemiosmotic mechanism with $Na^+$ as coupling ion [20,21] demonstrating that at least one of the reactions must catalyze electrogenic $Na^+$ transport across the membrane. Cytochromes or quinones were not detected in caffeate-grown cultures [17]. To unravel the enzymes involved in hydrogen-dependent caffeate

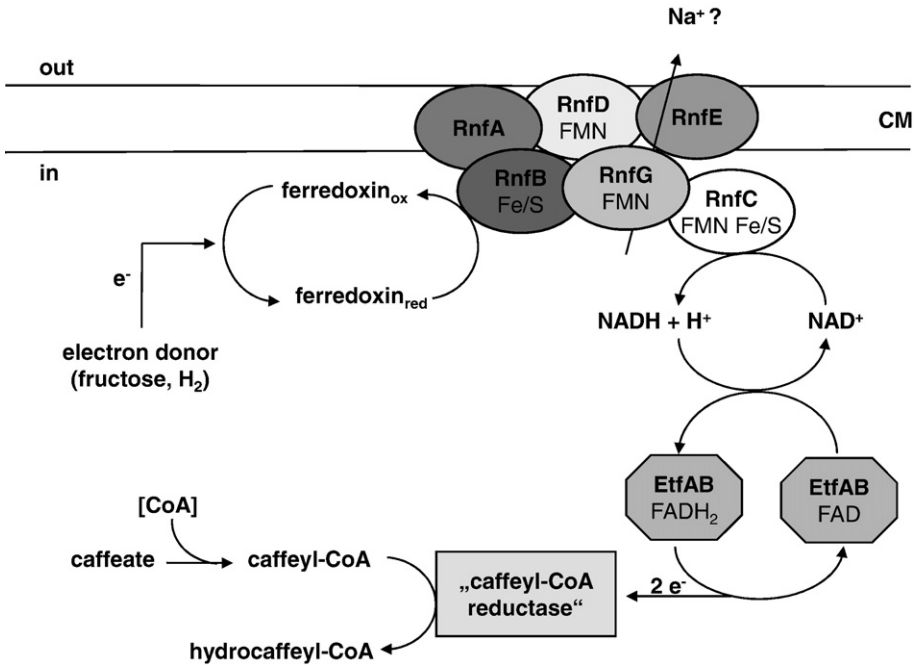


**Fig. 2.** Model for caffeate respiration in *A. woodii*. Pyruvate:ferredoxin oxidoreductase or hydrogenase generate reduced ferredoxin that is reoxidized by Rnf. The electron is then transferred via NADH + $H^+$ and the Etf complex to a hypothetical caffeyl-CoA reductase. Caffeate is likely activated prior to reduction. CM = cytoplasmic membrane.

reduction, subcellular systems were used, enzyme activities were measured and proteins involved were identified by a proteome approach. From these studies the following pathway was predicted: Hydrogen is oxidized by a soluble, iron-only hydrogenase that uses ferredoxin as an electron acceptor. Caffeate is probably activated to caffeyl-CoA prior to reduction. Electrons are derived from NADH + $H^+$ and transferred to caffeyl-CoA *via* an electron transferring flavoprotein (Etf) that is induced during growth on fructose + caffeate. The entire reaction sequence from $H_2$ to reduced ferredoxin and from NADH + $H^+$ to caffeyl-CoA was found in the soluble fraction which left the electron transfer from reduced ferredoxin to $NAD^+$ as the only membrane-bound and potentially $Na^+$-translocating reaction [22,23]. Indeed, a membrane-bound ferredoxin:$NAD^+$-oxidoreductase activity was found in washed membranes of *A. woodii* [23].

A candidate for the membrane-bound ferredoxin:$NAD^+$-oxidoreductase is the Rnf complex found to be encoded by several bacterial genomes [24–29]. Using primers derived from *rnfC* of *Clostridium tetani* we were able to amplify part of an *rnfC*-like gene of *A. woodii*. This was the first genetic hint to the presence of a Rnf complex in *A. woodii* [23]. Very recently, it was shown that the genome of *A. woodii* harbours six *rnf* genes that are organized in an operon. The operon is expressed, subunits of the enzyme were found in the cytoplasmic membrane by an immunological approach and the enzyme was partially purified from the cytoplasmic membrane by detergent solubilization, polyethylenglycol precipitation and sucrose gradient centrifugation [30]. Altogether, this is convincing evidence for the presence of a Rnf complex in *A. woodii*. A summary of the pathway of electron flow from various donors to caffeate is shown in Fig. 2.

### 2.2. The Rnf complex of A. woodii

The Rnf complex was first discovered in the purple nonsulfur bacterium *Rhodobacter capsulatus* [29]. Through analysis of defined insertion and deletion mutants of *R. capsulatus* seven genes were identified (*rnfABCDEGH*) that were required for nitrogen fixation (Rnf = *Rhodobacter* nitrogen *f*ixation). Bioinformatic analyses revealed that Rnf subunits are similar to subunits of the $Na^+$-translocating NADH:quinone-oxidoreductase (Nqr). Nqr was first found in the marine bacterium *Vibrio alginolyticus* [31], but the enzyme is widespread in nature and has attracted considerable interest [31–34]. Like Rnf, Nqr complexes are NADH dehydrogenases consisting of 6 subunits [35]. They mediate electrogenic $Na^+$-translocation that is coupled to electron flow from NADH to ubiquinone [36].

The Rnf complex of *A. woodii* is encoded by six genes. RnfC is predicted to be a soluble protein with a molecular mass of 47 kDa, but it was shown by Western blotting that it is located in the membrane [30]. It contains potential NADH- and FMN-binding sites [30] and twice the consensus sequence C-XX-C-XX-C-XXX-C-P indicating the presence of two [4Fe/4S] clusters [37]. RnfG (22.8 kDa) is a hydrophilic protein, but contains a hydrophobic stretch of 30 amino acids at the N-terminus, indicating that it might be anchored in the membrane. Indeed, as for RnfC, it could be shown that RnfG is localized in the membrane fraction of *A. woodii*. Furthermore RnfG was found to contain a potential FMN binding site [30]. The subunit is similar to NqrC. For NqrC of *V. alginolyticus* it was shown that it contains covalently bound FMN [38] and recently it was discovered that the same is true for RnfG from *Vibrio cholerae* [39]. RnfB (36.6 kDa) of *A. woodii* is a hydrophilic protein, but like RnfG it contains a stretch of 30 hydrophobic amino acids at the N-terminus, indicating that it may also be anchored in the membrane. The amino acid sequence of RnfB argues for the presence of 6 [4Fe/4S] clusters and it is similar to ferredoxin. RnfB of *R. capsulatus*, produced in *E. coli* was found to contain one [4Fe/4S] cluster [37]. RnfA, RnfD and RnfE are predicted to be transmembrane proteins. RnfA (21.4 kDa) has 7–9 predicted transmembrane helices, whereas RnfD (35.5 kDa) contains 8–9 and RnfE (21.5 kDa) 5–7. RnfD is similar to NqrB, and RnfA and RnfE are both similar to NqrD and NqrE. For RnfD from *V. cholerae* it was recently shown that it contains a covalently bound FMN [39]. In RnfD of *A. woodii* the same conserved residue for binding is present, so it is reasonable that it also contains a covalently bound FMN [30].

The overall similar subunit organization as well as the similarity of single subunits of Rnf to Nqr subunits has led to the idea that the Rnf complex of *A. woodii* mediates electrogenic $Na^+$ transport driven by electron transport from reduced ferredoxin to $NAD^+$. The difference in redox potential between ferredoxin ($E^{0\prime}$ = ca. −420 mV) and NADH ($E^{0\prime}$ = −320 mV) is rather small and may not exceed −100 mV equivalent to approx. −20 kJ/mol. Assuming an electrochemical ion potential of around −200 mV across the cytoplasmic membrane the

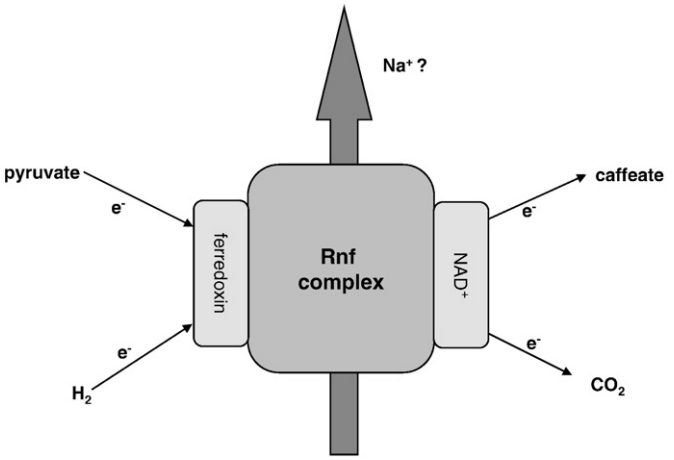


**Fig. 3.** Rnf may have a central role in the bioenergetics of *A. woodii*. Oxidation of pyruvate (glycolysis) or $H_2$ (chemolithoautotrophic growth) yields reduced ferredoxin. Electrons are wired to $NAD^+$ by Rnf thereby generating a $Na^+$-potential. Depending on the availability of electron acceptors, either $CO_2$ or caffeate is reduced.

free energy change of this reaction would allow for the translocation of only one ion across the membrane indicating that three mol of reduced ferredoxin must be oxidized to give the three ions required by the ATP synthase to synthesize one mol of ATP.

It should be stressed that there is no biochemical proof for ion export coupled to electron transport through any Rnf complex. However, our finding that caffeate respiration is coupled to ATP synthesis by a chemiosmotic mechanism with $Na^+$ as coupling ion and the finding that a ferredoxin:$NAD^+$-oxidoreductase was the only membrane-bound enzyme detected in the pathway of hydrogen-dependent caffeate reduction makes it likely that the Rnf complex of *A. woodii* is indeed a sodium ion pump. Purification and characterization of the enzyme is currently under way.

### *2.3. The Rnf complex, a potential coupling site also in the Wood–Ljungdahl pathway?*

Despite the still not answered question whether one of the enzymes catalyzing the carbon flow in the Wood–Ljungdahl pathway in $Na^+$-dependent acetogens is localized in the cytoplasmic membrane [14], the Rnf complex could also represent a (the) coupling site in the Wood–Ljungdahl pathway and thus be a coupling site in carbonate respiration (Fig. 3). The hydrogenase (during chemolithoautotrophic growth) and the pyruvate:ferredoxin-oxidoreductase (during heterotrophic growth) both generate reduced ferredoxin. Rnf may catalyze electron flow from reduced ferredoxin to $NAD^+$ which is then used as reductant in the Wood–Ljungdahl pathway. Three reductive steps leading from $CO_2$ to the methylated corrinoid-iron sulfur protein use NADH or NADPH as reductant. The possible involvement of the Rnf complex in the electron flow in the Wood–Ljungdahl pathway is an interesting idea that clearly needs to be further addressed by appropriate experiments.

## 3. Utilization of the sodium motive force in *A. woodii* by a $Na^+$ $F_1F_O$ ATP synthase

*A. woodii* is one of the rare cases of organisms that rely completely on a sodium motive force for its bioenergetics. It has a $Na^+$-driven flagella motor [40], a $Na^+$-driven ATP synthase and grows in the absence of a proton potential demonstrating that all growth-essential transport processes couple to $\Delta\mu Na^+$. The $Na^+$-driven ATP synthase has been studied intensively and attracted considerable interest [41–46].

### *3.1. The ATP synthase of A. woodii is a $Na^+$ $F_1F_O$ ATP synthase*

The overall structure of the ATP synthase determined by primary sequence comparisons [46–49], subunit composition analyses [45,50] and electron microscopy studies [51] revealed that it is similar to other $F_1F_O$ ATP synthases. Therefore, it is likely that the enzyme from *A. woodii* is in its function and molecular architecture similar to those of other organisms. A hypothetical model is depicted in Fig. 4. The central stalk is built by γ and three copies of each subunit α and β alternate around it. Subunit ε is part of the central stalk and connects it *via* subunit *c* with the membrane domain. In the membrane are subunit *a* (one copy), subunit *b* (two copies) and a ring of subunit *c*. That the $F_1F_O$ ATP synthase is a rotary device has been clearly shown [52–55]. The rotation of the *c* ring is most probably coupled to the ion flow through the membrane along the *a*–*c*-interface, so that this rotation finally leads to a rotation of the central stalk (subunit γ) [55–57]. Rotation of subunit γ within the $\alpha_3\beta_3$ headpiece results in the liberation of ATP from the β subunits [58]. Such a mechanism requires a stator, which is most likely built by subunit *b* and δ (Fig. 4). The rotor is made from multiple copies of subunit *c* that form a ring. The size of the ring varies from 10–15 subunits, depending on the species [41,59–64]. Each of the subunits has an ion binding site which is a conserved carboxylate (asp or glu) in helix two. In addition to the carboxylate, two more residues of subunit *c* are required to coordinate the $Na^+$ in $Na^+$ $F_1F_O$ ATP synthases [62].

### *3.2. The motor is a hybrid motor that contains $F_O$ and $V_O$-like c subunits*

*A. woodii* has multiple genes encoding *c* subunits that are all embedded in the $F_1F_O$ ATP synthase operon. This distinguishes it from all other known $F_1F_O$ ATP synthase operons. $AtpE_2$ (subunit $c_2$) and $AtpE_3$ (subunit $c_3$), which are 100% identical on the amino acid level, arose from an ancestral gene by a duplication event [46,47,49]. Only 18 substitutions appear on the DNA level. They have a molecular mass of 8.18 kDa and as their bacterial homologues, they are supposed to be organized in the membrane like a hairpin with two transmembrane helices connected by a polar loop. $atpE_1$ differs from $atpE_{2/3}$ with more than double the size (546 bp). On DNA level the first and second halves are 66% identical, which indicates a duplication of a precursor and following fusion of the two gene copies. The deduced molecular mass of $AtpE_1$ (subunit $c_1$) is 18.37 kDa with four predicted transmembrane helices arranged in two hairpins. However, it has only one ion binding site since the carboxylate is substituted by a

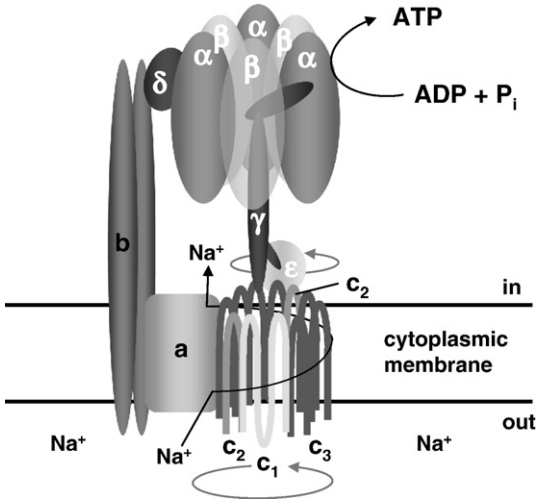


**Fig. 4.** Hypothetical structure of the $Na^+$ $F_1F_O$ ATP synthase from *A. woodii*. The model is based on molecular and biochemical data and comparisons to known high resolution structures of $F_1F_O$ ATPases. Arrows denote directions of rotation. The stoichiometry for $c_2/c_3$: $c_1$ is 9:1.

glutamine residue in hairpin two. The duplication of the *c* subunit gene *per se* is not of great consequence for the function of the ATPase since it was shown before that genetically engineered duplicated *c* subunits from *E. coli* are functional in $H^+$ transport and ATP synthesis [65,66]. A noticeable feature of $c_1$ is the fact that the ion-translocating residue is not conserved in helix two. A loss of one ion-translocating residue is also found in the "16-kDa *c* subunits" from eukarya, which goes along the fact that the $V_1V_O$ ATPases are not able to synthesize ATP *in vivo* [67,68]. The capability to synthesize ATP is directly dependent on the number of ions translocated per ATP synthesized. According to $\Delta G_p = -n \cdot F \cdot \Delta p$, a phosphorylation potential ($\Delta G_p$) of ~50 to 70 kJ/mol is sustained by the use of $n = 3$–4 ions/ATP at a physiological electrochemical ion potential of −180 mV ($\Delta p$). However, if the number of protons is lower, then ATP can no longer be synthesized. Taking into account three ATP-synthesizing or hydrolyzing centers and 12 $F_O$-like *c* subunits, this gives a stoichiometry of 4 ions/ATP. In contrast, six copies of the "16-kDa proteolipid" with one ion binding site in four transmembrane helices gives a stoichiometry of only 2 ions/ATP, which is too low to allow ATP synthesis. On the other hand, if the number of ions is low, the same $\Delta G_p$ can account for a much higher $\Delta p$, making the enzyme a better proton pump, a function required by the physiology of the eukaryotic cell [69]. In general, the smaller the number of carboxylates per ring, the lower is the coupling efficiency. Taking this into account it is now reasonable to assume that a cell could, depending on the cellular needs, alter the function of the ATPase between ATP synthesis and ATP hydrolysis by varying the number of proton (ion) translocating residues. This is a very attractive idea for *A. woodii*, which can grow by fermentation or by anaerobic respiration and has the genetic capacity to switch the subunit composition of its rotor. During fermentation the enzyme has to work as an ion pump generating the membrane potential whereas during autotrophic growth on $H_2 + CO_2$ it has to work as a synthase. The switch from pump to synthase could be performed by changing the ratio of $c_1/c_{2/3}$ [44].

This hypothesis was recently addressed. The ATP synthase was solubilized with β-D-dodecylmaltoside and further purified to apparent homogeneity by chromatographic techniques. The preparation contained, along with the $F_1$ subunits, the entire membrane-embedded motor with the stator subunits *a* and *b*, and the *c* ring. Immunological as well as biochemical analyses revealed the presence of subunit $c_1$ and subunit $c_{2/3}$ in the motor domain. After incorporation into liposomes, ATP synthesis could be driven by $\Delta\mu Na^+$ and $\Delta\Psi$, but not by $\Delta p$Na. This was the first demonstration that an ATPase with a $V_O$–$F_O$-hybrid motor is capable of ATP synthesis [43].

To determine whether both types of rotor subunits are present in the same *c* ring we have isolated and studied the composition of the *c* ring [41]. High-resolution atomic force microscopy (AFM) of 2D crystals revealed 11 domains, each one corresponding to two transmembrane helices. A projection map derived from electron micrographs, calculated to 5 Å resolution, revealed that each *c* ring contains two concentric, slightly staggered, packed rings, each composed of 11 densities, representing 22 transmembrane helices. The inner and outer diameters of the rings, measured at the density borders, are approximately 17 Å and 50 Å. Mass determination by Laser Induced Liquid Beam Ion Desorption (LILBID-MS) clearly gave the evidence that the *c* rings contained both types of *c* subunits. The stoichiometry for $c_2/c_3$: $c_1$ was determined to be 9:1. These analyses clearly demonstrated, for the first time, a $F_O$–$V_O$ hybrid motor in an ATP synthase. However, the stoichiometry was independent of the carbon source and the $Na^+$ concentration of the medium which argues against a regulation of activity by changing subunit composition.

## 4. Concluding remarks

Acetogens are truly fascinating organisms. They can thrive in anoxic environments using a wide variety of different electron donors, organic as well as anorganic, for carbonate respiration. Not only carbonate, but also other electron acceptors are used by acetogens. The fact that acetogens can grow non-acetogenically was an outstanding observation. It does not only explain the ecologically important fitness of acetogens in their environment but also enabled the study of electron transport chains other than those involved in carbonate reduction.

Analyses of electron flow during caffeate respiration revealed the Rnf complex of *A. woodii* as the potential coupling site. However, this still has to be demonstrated experimentally. An open question is also whether and how caffeate is activated prior to reduction. If caffeyl-CoA is the substrate the question arises whether the Etf-mediated caffeyl-CoA reduction is coupled to ferredoxin reduction, as recently suggested for crotonyl-CoA reduction in *Clostridium kluyveri* [70,71]. This would give some additional energy conservation *via* Rnf. From the finding that Rnf is present also in carbonate respiring cells it was concluded that Rnf is also a (the) coupling site in acetogenesis. This highly attractive but also speculative idea certainly needs further experimental attention.

Although the analyses of the biochemistry and bioenergetics have reached a highly sophisticated level, a genetic system that would be important to address cellular functions of proteins such as Rnf or the $V_O$-like rotor subunits is still missing. The genome of *M. thermoacetica* has been published recently [72], and the one of *A. woodii* is in progress. Therefore, establishing a genetic system for these two acetogenic model systems should have highest priority in the near future. This could not only address pathways for carbon and electron flow but also the molecular basis of regulation of electron acceptor usage.

## Acknowledgments

This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB472 and MU801/12) and the "Center for Membrane Proteomics" (fellowship to Silke Schmidt).

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