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Raamsdonk, L.M.

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Chapter 3

Contribution of $F_1 \cdot F_0$ ATP synthase in *Saccharomyces cerevisiae* cells to energetics and glucose metabolism

In collaboration with Viktor Boer, Jan A. Berden, Hans V. Westerhoff, M. Joost Teixeira de Mattos, and Karel van Dam

In this paper we report on how carbon and energy metabolism of *Saccharomyces cerevisiae* change in response to a deletion of the gene encoding the (catalytic) β-subunits of the $F_1 \cdot F_0$ ATP synthase. The knockout strain is unable to grow on non-fermentable carbon sources. When grown batch-wise on glucose no changes in the concentration of intracellular metabolites such as G6P, pyruvate or even ATP, ADP and AMP were detected. The growth yields of both the wild-type strain and the *atp2* deletion strain were similar to that of the wild-type strain growing anaerobically, i.e. 0.10 g DRW x g^{-1} glucose. The deletion of the β-subunits reduced the *in vivo* respiration by some 10 %, increased the production of ethanol and glycerol by approximately 15%, and decreased the specific growth rate by almost 20 %. This shows that when *S. cerevisiae* grows fermentatively, the$F_1 \cdot F_0$ ATP synthase does play a significant role in metabolism and growth although this has little or no implication for growth yield.

In a glucose-limiting chemostat cultures, where the wild-type *S. cerevisiae* strain switched to the respiration mode, the *atp2* deletant differed dramatically from the wild type. At the relatively low dilution rate (D) of 0.1 h^{-1}, the specific glucose consumption rate was more than 5 times larger in the *atp2* deletion strain than in the wild-type strain. The biomass yield of the mutant had remained the same as under the glucose excess conditions whilst the growth yield of the wild-type strain increased 4.5 fold. The specific oxygen consumption rate in the *atp2* deletion strain was higher than in the wild-type strain at this dilution rate; had the

ATP synthase been present this respiration would have sufficed to energize the growth. Growth of the mutant was fermentative, 80% of the catabolized glucose being converted to ethanol.

3.1 Introduction

Under anaerobic conditions the yeast *S. cerevisiae* depends solely on substrate level phosphorylation in glycolysis for its ATP production, as no oxygen is present to act as the terminal electron acceptor in the respiratory chain. On the other hand, when *S. cerevisiae* grows aerobically on a non-fermentable carbon source (*e.g.*, ethanol, lactate or glycerol), ATP mainly is derived from the oxidative phosphorylation in the mitochondria. A fermentable carbon source (*e.g.*, galactose, sucrose or glucose) can either be fermented to ethanol or completely oxidized to water and CO_2, obtaining ATP from substrate level phosphorylation or both substrate level phosphorylation and oxidative phosphorylation, respectively. However, fermentation of, for example, glucose yields only 2 ATP whereas complete oxidation of glucose is much more productive and yields approximately 20 ATP per glucose molecule consumed.

Up to this point, *S. cerevisiae* is not that special. However, upon addition of glucose to growing cells, glucose repression or more generally, catabolite repression sets in (for reviews see: [29,63,101,211]). This phenomenon implies the down-regulation of the transcription of genes involved in the tricarboxylic acid (TCA) cycle, the biogenesis of mitochondria and of genes whose products contribute to the metabolism of carbon sources other than glucose or fructose. The result is a preferred consumption of glucose over other carbon sources and the conversion of this glucose to mainly ethanol and glycerol. Under glucose repressing conditions, most ATP is therefore produced by substrate level phosphorylation in glycolysis, with the consequence of a low growth yield [117]. *S. cerevisiae* is one of the few yeasts that exhibits this aerobic fermentation; such yeasts are known as Crabtree positive. Most other yeasts, *e.g.*, *Kluyveromyces lactis* are Crabtree negative and will not ferment in the presence of oxygen and, consequently, grow with a much higher growth yield on glucose [216]. An implication is that for a Crabtree positive yeast such as *S. cerevisiae* it should be relatively straightforward to grow aerobically on sugars without functional oxidative phosphorylation. The mitochondrial $F_1{\cdot}F_0$ ATP synthase is the terminal complex in oxidative phosphorylation and plays a central role in free-energy transduction. $F_1{\cdot}F_0$ ATP synthase consists of a proton-conducting F_0 unit and a catalytic F_1 unit both units consist of several subunits. The catalytic sites are located on the β-subunits of the F_1 unit, encoded by the *ATP2* gene. The above implication now translates into the prediction that

S. cerevisiae should be little effected by a deletion of the beta subunit when growing on excess glucose. It is this prediction which we test in the present paper.

The analogous test in *Escherichia coli* has led to some surprises: a deletion of the operon comprising the genes encoding F_1F_0 ATP synthase, resulted in many changes, including a decrease in specific growth rate on glucose by approximately 25%, and a strong decrease in growth yield by approximately 45% [96]. The respiration rate was increased by 40%, the proton motive force by approximately 20%, and the expression of certain cytochromes by some 80% while the acetate production was doubled. Furthermore, the catabolized carbon flux doubled and the flux through the TCA cycle increased by 50%. Thus, *E. coli* solves the absence of oxidative phosphorylation by increasing both the oxidative and the fermentative flux so that substrate level phosphorylation coupled to both routes increases. The complementation of the decreased ATP production by the increase of the flux is obviously not sufficient, since the specific growth rate decreased by 25% and ATP/ADP ratio decreased by more than 60%.

Here we investigated the contribution of F_1F_0 ATP synthase to metabolism in *S. cerevisiae* under glucose-repressed conditions, and for comparison, also under glucose derepressed conditions, *i.e.*, in glucose-limited continuous cultures. We report that under glucose excess conditions deletion of the ATPase does have significant effects on glucose and energy metabolism, however, without affecting the growth yield to any extent. The growth yield of derepressed *S. cerevisiae* depended quite strongly on the ATP synthase. *S. cerevisiae* appears to be casual about its energy metabolism, being able to greatly accelerate its glycolytic flux when sufficient glucose is present.

3.2 Material and methods

Strains and their constructions

The strains used in this work are listed in Table 3.1. CEN.PK113-5D is a wild-type *Saccharomyces cerevisiae* strain and obtained from Dr. Kötter in Frankfurt, Germany. The strain LRY11A was constructed by knocking out the *ATP2* gene in the wild-type strain CEN.PK102-3A by homologous recombination. This was attained as follows: the plasmid pMBYDL2 (a kind gift from Dr. D. Mueller) [141] was digested with *EcoR1/SacI* to obtain a *LEU2* selection marker flanked with the 5' and 3' end of the *ATP2* gene. The fragment of approximately 3.8 kb was isolated from gel transformed in the *ATP2* locus of the genome of competent CEN.PK102-3A cells as described by [68]. After 2 h of cultivation in YPD medium (1% yeast extract, 2% peptone, 2% glucose), the transformed cells were plated on solid YNB medium lacking leucine

and incubated at 30° C. The leucine positive isolates were replica-plated on medium with glucose and on medium with ethanol as the sole carbon source. The strains that were able to grow on glucose but unable to grow on ethanol were tested for proper replacement of the *ATP2* locus by Southern blot. For Southern blotting, the chromosomal DNA of 3 selected mutants and CEN.PK102-3A was isolated and digested with *ApaI* and run on gel and blotted. The *KpnI/SacI* fragment of the *ATP2* fragment of plasmid pT7-ATP2 (0.6 kb)[32] was used as a probe. The wild-type strain yielded the expected fragment of approximately 4.3 kb whereas the mutant yielded the expected fragment of 5.5 kb. Additionally, the selected mutant strain, LRY11A, revealed no detectable mitochondrial ATPase activity.

Strains	Genotype	Ref.
CEN.PK113-5D	*MATa ura3-52 HIS3 LEU2 TRP1 MAL2-8c SUC2*	Dr. P. Kötter, Frankfurt
CEN.PK113-3A	*MATa ura3-52 HIS3 leu2-3 TRP1 MAL2-8c SUC2*	Dr. P. Kötter, Frankfurt
LRY11A	*MATa ura3-52 HIS3 leu2-3 TRP1 MAL2-8c SUC2 atp2Δ::LEU2*	This work

Table 3.1: Used strains

Batch growth conditions

Cells were grown in batch in an orbital incubator at 250 rpm and 30°C in a medium containing 2% (w/v) glucose (unless indicated differently), 0.17% (w/v) Yeast Nitrogen Base without amino acids and ammonium sulphate (Difco), 0.5% (w/v) $(NH_4)_2SO_4$ and 0.1 M potassium phthalate at pH 5.0 including the required addition of uracil (40 mg/l). Growth was monitored by measuring the optical density at 600 nm.

Chemostat growth conditions

The mineral medium for continuous cultures contained per litre: 5g $(NH_4)_2SO_4$, 3g KH_2PO_4, 0.5g $MgSO_4 \cdot 7H_2O$, 15 mg EDTA, 4.5 mg $ZnSO_4 \cdot 7H_2O$, 0.3 mg $CoCl_2 \cdot 6H_2O$, 1 mg $MnCl_2 \cdot 4H_2O$, 0.3 mg $CuSO_4 \cdot 5H_2O$, 4.5 mg $CaCl_2 \cdot 2H_2O$, 3 mg $FeSO_4 \cdot 7H_2O$, 0.4 mg $NaMoO_4 \cdot 2H_2O$, 1 mg H_3BO_3, 0.1 mg KI, and 0.025 ml silicone antifoam (BDH). Final vitamin concentrations per litre: 0.05 mg biotin, 1 mg calcium pantothenate, 1 mg nicotinic acid, 25 mg inositol, 1 mg thiamine HCl, 1 mg pyridoxine HCl, 0.2 mg para-aminobenzoic acid, and 40 mg uracil. The medium was prepared and sterilized as described by [224]. For chemostat cultivation, the glucose concentration in the medium was 7.5 $g.l^{-1}$ for the *atp2* deletion strain and 2.5 $g.l^{-1}$ for the parent strain.

The aerobic chemostat cultivation was performed at 30°C in laboratory fermentors, at a

stirrer speed of 1250 rpm. The pH was kept at 5.0 by the automatic addition of 2 M KOH. The fermentor was aerated with 1 vessel volume per minute. A steady state was presumed after at least 5 volume changes of growth without changes in any of the growth conditions.

CO_2 production and O_2 consumption measurements

Oxygen consumption and carbon dioxide production in the continuous cultures were determined by passing the gas from the fermentor through an oxygen analyser (Taylor Servomex Type OA 272) and a carbon dioxide analyser (Servomex IR Gas Analyser PA 404).
Oxygen consumption by the batch cultures was measured with an oxygen electrode in a stirred thermostatically controlled chamber at 30°C. Samples were taken from the culture, briefly aerated and one ml was pipetted in the chamber.

Metabolite, dry weight and protein determinations

Samples for protein determination were taken by spinning down 1 ml of culture and dissolving the pellet in 1 ml of 1N NaOH. The determination was performed as described in Bergmeyer [13] by the method of Lowry [125], using bovine serum albumin (fatty-acid free) as a standard. The intracellular metabolites in the batch-wise growing cells were determined by quenching cells in cold (-40°C) 50% (w/v) methanol (final concentration) [39] and extracting the metabolites as described in [71]. The intracellular metabolite concentration in the continuous cultures was determined by rapid quenching of 600 µl culture in 100 µl 35% PCA (v/v) and by neutralizing with 145 µl 2 M K_2CO_3 at 0°C after approximately 15 min. The suspension was spun down and the intracellular metabolites were measured in the supernatant. For the determination of the extracellular metabolites, 100 µl 35% PCA (v/v) was injected to 1 ml of culture supernatant. Samples were partially neutralized after 20 minutes at 0 °C with 55 µl 7N KOH. After centrifugation the supernatant was filtered and analysed for glucose, ethanol, glycerol, acetate and pyruvate by HPLC (column: Phenomenex type Rezex Organic Acid, eluent: 7.2 mM H_2SO_4 at 40°C). Both protein and metabolites were measured on a COBAS-BIO and COBAS-FARA automatic analyser (Roche) according to Bergmeyer [13].

Dry weight of the cells was determined in duplicate by spinning down 10 ml cells at 4°C and washing once. The pellet was dried overnight at 110 °C and weighed the next morning. For the batch cultivations the glucose and biomass concentration were determined directly after inoculation and at right before harvesting again. The biomass yield was calculated from these data.

Mitochondrial ATPase activity

For the determination of the $F_1 \cdot F_0$ ATP synthase activity 10 ml of culture was centrifuged for 5 min at 4000 x g at 4°C. The pellet was resuspended in 200 µl buffer containing 500 mM mannitol, 1 mM ATP, 2 mM EDTA, 0.2% BSA (w/v), 10% methanol, 10 mM ε-aminocaproic acid in 0.1 M Tris/HCl (pH 7.5) and extracted by vigorous shaking for 30 min with 0.2 g glass beads at 4°C [214]. To inhibit protease activity 1 µM PMSF (final concentration) in DMSO was added. The ATPase activity was measured spectrophotometrically following NADH oxidation using a coupled system with lactate dehydrogenase and pyruvate kinase as described in [200]. The oxidation was measured with and without the addition of azide as the specific inhibitor of the $F_1 \cdot F_0$ ATP synthase. Mitochondrial ATPase activity is defined as the activity corrected for the azide-resistant activity.

Calculations

Calculation of q_{ATP}:

Since the *atp2* deletion strain can only produce ATP *via* substrate level phosphorylation, the q_{ATP} (mmol ATP x (g dry weight)$^{-1}$ x h^{-1}) can be calculated. For each ethanol (EtOH), acetate (HAc) or pyruvate (Pyr) formed, 1 ATP is produced. The production of 1 glycerol costs 1 ATP. For each round of TCA cycle: 1 ATP is formed and the cycle forms 3 CO_2, including the CO_2 released by the conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase. The ATP formed in the TCA cycle is calculated by subtracting the CO_2 formed by fermentation from the total CO_2 produced, divided by 3: $q_{EtOH} + q_{HAc} + q_{Pyr} - q_{Glycerol} + (q_{CO2} - q_{EtOH} - q_{HAc})/3$

Calculation of Y_{ATP}:

The yield of ATP or Y_{ATP} (g DRW x mol^{-1} ATP) is calculated by: D x $(q_{ATP})^{-1}$. This results in an average value of 11 g DRW x mol^{-1} ATP for all dilution rates measured (of which only D = 0.1 h^{-1} is shown here). We assume that the Y_{ATP} is the same in the wild-type strain which enables us to calculate the q_{ATP} for the wild-type strain by: D x $(Y_{ATP})^{-1}$ x 1000 = 1000xD/11. In the *atp2* deletion strain the q_{ATP}^{Total} equals the rate of ATP formed in the substrate level phosphorylation (SLP) q_{ATP}^{SLP} whereas no ATP is formed by oxidative phosphorylation (Oxphos) thus, $q_{ATP}^{Oxphos} = 0$. For the wild-type strain the q_{ATP}^{SLP} can also be calculated at D=s lower than the critical µ. Oxidation of one glucose molecule yields 2 ATP in glycolysis plus 2 ATP in the TCA cycle minus the glucose fraction that is consumed for biomass formation, thus: $q_{ATP}^{SLP} = 4 \times q_{glc} \times (1 - Y_{biomass})$. The $q_{ATP}^{Oxphos} = q_{ATP}^{Total} - q_{ATP}^{SLP}$.

The carbon balance of the wild-type added up to 104% whereas the carbon balance of the mutant added up to 90%.

3.3 Results and discussion

Batch cultures

The constructed *atp2* deletion strain was devoid of any detectable level of $F_1 \cdot F_0$ ATPase activity whereas the parent strain exhibits low, glucose-repressed levels of mitochondrial ATPase activity in the presence of excess glucose (Table 3.2). Indeed this mutant was not capable of growing on non-fermentable carbon sources such as ethanol, lactate or glycerol and grew poorly on non-repressible carbon sources such as galactose (data not shown).

To test the expectation that the ATP synthase is of minor significance for Crabtree positive yeasts, the *atp2* deletion strain and the wild-type were grown batch-wise on different glucose concentrations, varying from 0.5% to 5%. Physiological properties such as specific respiration rate, specific growth rate and production of fermentative products, were compared. The specific growth rate of the *atp2* deletion strain grown aerobically on glucose, was almost 20% lower than that of the parent strain (0.39 vs. 0.32 h^{-1}) (Table 3.2) and slightly higher than the anaerobic specific growth rate of its parent (0.30 h^{-1}) [218]. Despite the absence of ATP synthase activity and the affected specific growth rate, the concentrations of the intracellular metabolites, glucose 6-phosphate, fructose 6-phosphate and pyruvate, were identical between mutant and wild-type cells including the adenine nucleotides such as ATP and ADP, at glucose concentrations of 1% and higher (Table 3.2). This shows that $F_1 \cdot F_0$ ATP synthase has no control on the intracellular ATP and ADP concentration in yeast cells but has control on the specific growth rate. This is in agreement with the lack of control found on ATP and ADP concentration in strains with decreased $F_1 \cdot F_0$ ATP synthase activity when grown on non-fermentable carbon sources and the substantial control on the specific growth rate (see Chapter 4 of this thesis). The results strongly differ from those in *E. coli*.

The ethanol and glycerol concentrations in the *atp2* strain were increased by approximately 10%, revealing a slightly higher fermentative flux. The elevated glycerol production and ethanol production is more pronounced at glucose concentrations below 2% , due to partial derepression of the oxidative metabolism in the parent strain. At 0.5% glucose, $F_1 \cdot F_0$ ATP synthase was significantly derepressed in the wild type cells: the azide- sensitive ATPase activity in the parent is approximately 0.3 mmol ATP x min^{-1} x (g protein)$^{-1}$.

			Intracellular metabolites (mM)				Yield	ATPase	O_2	Extracellmetab (mM)	
	µmax (h^{-1})	Glc (%)	G6P	ATP	ADP	ATP/ ADP	(g DRW/ g glc)	(mmol /min/ g protein)	(µmol/ min/ g protein)	Gly	EtOH
Wt	0.39	0.5§	1.6	4.6	1.2	4.0	0.13	0.3	123	2.2	33
		1	2.6	4.4	1.6	2.8	0.1	0.2	71	4.8	44
		2	2.4	3.8	1.4	2.7	0.1	0.1	64	6.1	51
		3	1.6	3.6	1.6	2.3	0.1	0.1	51	7.0	50
		4	2	3.5	1.4	2.4	0.09	0.1	45	6.7	48
		5	1.5	2.4	0.8	2.8	0.1	0.1	45	7.1	45
atp2Δ	0.32	0.5*	-	-	1.4	-	0.1	nd	44	6.2	46
		1	2.6	3.7	1.4	2.6	0.09	nd	41	-	-
		2	2.1	3.1	1.2	2.6	0.09	nd	42	7.9	56
		3	2.2	3.4	1.2	2.8	0.1	nd	42	8.0	58
		4	3.1	3.7	1.3	2.8	0.08	nd	41	7.4	50
		5	1	2.3	0.9	2.6	-	nd	39	7.8	55

Table 3.2.: Properties of a parent strain and an *atp2* deletion strain grown in batch on glucose. Glc = glucose; G6P = glucose-6-phosphate; DRW = dry weight; Gly = glycerol and EtOH = ethanol; § = At diauxic shift at time of sampling; * = Glucose exhausted at time of sampling. nd = not detectable. The extracellular metabolite concentrations are determined at an $OD_{600} = 1$.

For comparison, completely derepressed wild-type cells, *e.g.*, growing on ethanol, reveal a mitochondrial ATPase activity of approximately 1 mmol ATP x min^{-1} x (g protein)$^{-1}$ (data not shown) whereas fully repressed activity is approximately 0.1 mmol ATP x min^{-1} x (g protein)$^{-1}$ (Table 3.2). At 1% glucose, respiration in the wild-type cells was increased, but surprisingly this did not increase the growth yield significantly, which remained 0.10 g DRW/g glucose. This value is similar to the yield of biomass of an anaerobic wild-type culture [218].

Contribution of oxidative phosphorylation to ATP production

The respiration rate in the *atp2* deletion mutant was low and constant at all glucose concentrations tested (Table 3.2). This rate of respiration represents State-4 respiration since the mitochondrial proton motive force, maintained by the respiratory chain, is not used for ATP synthesis. This respiration rate was not higher than in the wild-type strain, suggesting that there was no increased proton conduction by assembled F_0-subunits in the mitochondrial inner membrane unconnected to F1 subunits. This is in line with the observation in an *ATP5* mutant (encoding the OSCP subunit of F_1F_0 ATP synthase) the F_0 is not assembled, nor embedded in the membrane when F_1 is not attached [171]. The respiration in the *atp2* deletion strain did not increase with decreased extracellular glucose concentration, at least not to the extent it did in the wild-type cells, where the increase in ATPase activity suggests increased biogenesis of mitochondria. The phenomenon of derepression, therefore, is not observable in the mutant.

At glucose concentrations higher than 3% the respiration rate of the parent strain seems fully repressed but remained approximately 10% higher than that of the *atp2* deletion strain (Table 3.2). Below 3% glucose, the respiratory chain complexes became already partly derepressed in the parent strain, as deduced from the increase in the specific oxygen consumption rate. This contradicts the general assumption that metabolism of *S. cerevisiae* is fully repressed at 2% glucose. The onset of glucose derepression during glucose consumption in a batch culture appears to occur in phases, with the respiratory chain as one of the first (see this chapter) and gluconeogenesis as one of the last targets to be derepressed [248].

Despite the similar yield of the *atp2* deletion strain and its parent, the specific growth rate of the *atp2* deletion strain on glucose was decreased by approximately 18%. This suggests that the ATP supply in the *atp2* deletion strain is not fast enough to maintain the specific growth rate at wild-type level. The 10% higher flux through the respiratory chain and the slightly lower glycerol production at high glucose in the wild-type strain compared to the *atp2* deletion strain apparently enables the ATP supply to account for the higher growth rate in spite of the unchanged growth yield. The lower flux in the mutant can well be explained by the lower intracellular pH in the *atp2* deletion strain slowing down metabolic fluxes (see chapter 4 of this

thesis). The intracellular pH of glucose growing cells was 6.5 in the mutant and 6.9 in the wild-type strain (data not shown).

Decreased respiration rate

The decreased oxygen consumption in the *atp2* deletion strain compared to its parent cannot be caused by the (partial) loss of mitochondrial DNA since rho^0 and rho^- mutations have been shown to be lethal in the absence of functional mitochondrial ATPase activity in both humans [26] and *S. cerevisiae* [33]. In wild-type yeast cells the respiratory chain maintains the proton motive force under aerobic conditions while under anaerobic conditions as well as in rho-mutants, F_1F_0 ATPase activity is essential to maintain the proton motive force over the mitochondrial inner membrane.

In contrast to *E. coli* [96], the flux through the respiratory chain in the *atp2* deletion strain was shown to be sensitive to the back pressure of the proton motive force since uncoupling of the pmf increased respiration (see chapter 4 of this thesis). Moreover, the F_1F_0 ATP synthase deletion strain of *E. coli* used the increased flux through respiration to oxidise the formed NADH to NAD^+. In *S. cerevisiae*, the reduced respiration rate combined with the increased glycerol production in the *atp2* deletion strain may indicate that the NAD^+ pool is replenished by glycerol production rather than by the respiratory chain.

Chemostat cultures

For comparison we also studied a situation where wild type *S cerevisiae* does engage in oxidative phosphorylation. In Table 3.3 properties of aerated glucose-limited chemostat cultures are listed at a dilution rate (D) of 0.1 h^{-1}. Similar to the batch cultures, the concentrations of the intracellular metabolites such as glucose-6-phosphate, fructose-6-phosphate, pyruvate, ATP, ADP and AMP were unchanged in the *atp2* deletion strain compared to the parent strain (data not shown).

Under these conditions the growth yield of the *atp2* deletion strain was lower by a factor 5 and the glucose flux was 5 fold higher as compared to the parent strain. Even at this low D value the flux in the mutant was not largely oxidative since glycerol and ethanol could be detected. Another indicator for the relative distribution of fluxes over the oxidative and fermentative routes is the respiratory quotient (RQ), which is the ratio of CO_2 production to O_2 consumption. If in this case glucose is fully oxidised, the RQ will equal 1; when glucose is also fermented the RQ increases until there is no flux through the respiratory chain. The RQ at a D of 0.1 h^{-1} was 3, whereas for the parent strain the RQ was 1 (Table 3.3) showing the mainly fermentative growth of the *atp2* mutant under glucose limiting culture conditions.

	wt	*atp2Δ*		wt	*atp2Δ*
$q_{glucose}$	1.1	5.8			
$q_{biomass}$	1.0	1.0	**RQ**	1	3
q_{EtOH}	0	6.0	Y_{ATP}	11	11
$q_{glycerol}$	0	0.1	q_{ATP}^{Total}	9	9
q_{HAc}	0	0.3	q_{ATP}^{SLP}	2.5	9
$q_{Pyruvate}$	0	0.28	q_{ATP}^{Oxphos}	6.5	0
q_{O2}	3.1	4.4	**P/O**	1.1	0
q_{CO2}	3.6	13	**Yield**	0.46	0.08

Table 3.3: Properties of the parent strain and the *atp2* deletion strain grown in an aerated glucose limited chemostat culture at a dilution rate of 0.1 h^{-1}. The flux (q) in mmol x g^{-1} drw x h^{-1}. The yield (Y) in g biomass x g^{-1} glucose. The respiratory quotient (RQ) = CO2 / O_2. The q_{ATP}^{Total} is the total flux of ATP production. The q_{ATP}^{SLP} is the ATP production rate by substrate level phosphorylation. q_{ATP}^{Oxphos} is the ATP production rate by oxidative phosphorylation. P/O is the q_{ATP}^{Oxphos} /(q_{O2}*2)

Despite the non-functional oxidative phosphorylation, the oxygen consumption rate at a D of 0.1 h^{-1} was higher in the *atp2* deletion strain than in the parent strain. This may be caused by a higher leak over the mitochondrial membrane or by an increased slip in the respiratory chain increasing the state 4 respiration. The wild-type strain can grow at a lower (State 3) respiration rate since the ATP synthesis by oxidative phosphorylation is enough to grow with a μ of 0.1 h^{-1}. The calculated P/O ratio for the parent strain was approximately 1.1 and the *in vivo* yield of ATP was 11 g DRW x (mol ATP)$^{-1}$ both in the parent and *atp2* deletion strain which are acceptable values [223,225]. The P/O ratio of the *atp2* deletion strain was 0 since the oxidative phosphorylation was not functional. The *atp2* deletion strain exhibited a lower maximal specific growth rate in the chemostat (0.18 h^{-1}) than in batch (0.32 h^{-1}) whereas the parent strain had the same maximal specific growth rate in both cases (0.39 h^{-1}). This might be related to the differences in growth medium for the batch and continuous cultures, in addition to a possibly increased demand of the mutant for certain vitamins or other nutrients.

The observed decreased growth and respiration in addition to the increased fermentation in the *atp2* deletion strain compared to the wild-type strain shows that F_1F_0 ATP synthase plays

a significant role in metabolism even under fully glucose repressing conditions.

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