Fermentation and alternative respiration compensate for NADH dehydrogenase deficiency in a prokaryotic model of DJ-1-associated Parkinsonism

Nadia Messaoudi, Valérie Gautier, Julien Dairou, Mouhad Mihoub, Gaëlle Lelandais, Philippe Bouloc, Ahmed Landoulsi and Gilbert Richarme

Correspondence
Gilbert Richarme
richarme@paris7.jussieu.fr

Stress Molecules, Institut Jacques Monod, Université Paris 7, 2 place Jussieu, 75005 Paris, France
Université Paris Diderot, Sorbonne Paris Cité, Unité de Biologie Fonctionnelle et Adaptative (BFA), UMR 8251, CNRS, Bioprofiler Facility, 75205, Paris, France
Laboratoire de Signalisation et Réseaux de Régulations Bactériens, UMR 8621, Institut de Génétique et Microbiologie, Orsay, France
Laboratoire de Biochimie et Biologie Moléculaire 03/UR/0902, Faculté des Sciences de Bizerte, Zarzouna 7021, Bizerte, Tunisia

YajL is the closest prokaryotic homologue of Parkinson’s disease-associated DJ-1, a protein of undefined function involved in the oxidative stress response. We reported recently that YajL and DJ-1 protect cells against oxidative stress-induced protein aggregation by acting as covalent chaperones for the thiol proteome, including the NuoG subunit of NADH dehydrogenase 1, and that NADH dehydrogenase 1 activity is negligible in the \( yajL \) mutant. We report here that this mutant compensates for low NADH dehydrogenase activity by utilizing NADH-independent alternative dehydrogenases, including pyruvate oxidase PoxB and \( \delta \)-amino acid dehydrogenase DadA, and mixed acid aerobic fermentations characterized by acetate, lactate, succinate and ethanol excretion. The \( yajL \) mutant has a low adenylate energy charge favouring glycolytic flux, and a high NADH/NAD ratio favouring fermentations over pyruvate dehydrogenase and the Krebs cycle. DNA array analysis showed upregulation of genes involved in glycolytic and pentose phosphate pathways and alternative respiratory pathways. Moreover, the \( yajL \) mutant preferentially catabolized pyruvate-forming amino acids over Krebs cycle-related amino acids, and thus the \( yajL \) mutant utilizes pyruvate-centred respiro-fermentative metabolism to compensate for the NADH dehydrogenase 1 defect and constitutes an interesting model for studying eukaryotic respiratory complex I deficiencies, especially those associated with Alzheimer’s and Parkinson’s diseases.

INTRODUCTION

YajL belongs to the PfpI/Hsp31/DJ-1 superfamily which includes chaperones (Quigley et al., 2003; Sastry et al., 2002), peptidases (Malki et al., 2005) and the Parkinson’s disease-associated protein DJ-1 (Canet-Avila et al., 2004; Cookson, 2005). The crystal structures of YajL and DJ-1 are strikingly similar (Wilson et al., 2003, 2005), and their backbone structures are essentially identical (0.9 Å Cα root-mean-square deviation), suggesting that they have similar functions.

DJ-1 is involved in the cellular response to oxidative stress, and has been suggested to function as a weak protease (Lee et al., 2003), an oxidative stress-activated chaperone (Le et al., 2012; Shendelman et al., 2004; Zhou et al., 2006), an atypical peroxiredoxin-like peroxidase (Andres-Mateos et al., 2007; Canet-Avila et al., 2004), a stabilizer of the antioxidant transcriptional regulator Nrf2 (Clements et al., 2006), an apoptosis inhibitor via interaction with Daxx (Junn et al., 2005), a transcriptional or translational regulator of gene expression (Cookson, 2005; van der

Received 16 June 2015
Revised 7 September 2015
Accepted 11 September 2015
Brug et al., 2008) and a regulator of uncoupling protein expression affecting the production of reactive oxygen species (Guzman et al., 2010).

Similar to DJ-1, YajL protects cells against oxidative stress and oxidative stress-induced protein aggregation, possibly through its chaperone function and control of gene expression (Kthiri et al., 2010a, b). We reported recently that YajL and DJ-1 function as covalent chaperones that, upon oxidative stress, form mixed disulfides with proteins containing essential cysteines for catalysis, such as glyceraldehyde 3-phosphate dehydrogenase and peroxidases, or FeS cluster binding, such as aconitases and the NuoG subunit of NADH dehydrogenase 1 (Le et al., 2012). Moreover, YajL and DJ-1 preferentially form mixed disulfides with sulfenylated proteins and protect cells against protein sulfenylation (Gautier et al., 2012). The specific interaction of YajL with the NuoG subunit of NADH dehydrogenase is likely responsible for the NADH dehydrogenase 1 defect of the yajL mutant, which can be rescued by YajL- and DJ-1-overproducing plasmids (Le et al., 2012). These results suggest that DJ-1 may be involved in biogenesis and stress protection of mitochondrial complex I (Danielson & Andersen, 2008). Respiratory chain abnormalities in cells of patients with Parkinson disease have been reported (Bindoff et al., 1989), and complex I inhibitors such as rotenone and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) produce a Parkinson-like syndrome in both humans and primates, identifying complex I as a major target in Parkinson’s disease. Moreover, the genetic causes of Parkinson’s disease highlight the importance of mitochondrial dysfunction: parkin, PINK1, DJ-1, LRRK2 and HTRA2 localize to the mitochondria (Schapira & Gegg, 2011), and their deficiency induces mitochondrial defects that contribute to oxidative stress-induced cell death (Irrcher et al., 2010).

The importance of complex I deficiencies in the aetiology of Parkinson’s disease, the specific involvement of YajL and DJ-1 in its biogenesis, and the scarcity of results describing the metabolic characteristics of DJ-1-deficient cells led us to investigate the metabolism of the yajL mutant to understand how cells compensate for NADH dehydrogenase 1 deficiency.

**METHODS**

**Bacterial strains and plasmids.** The yajL-deficient strain was constructed by Dr H. Mori (Nara Institute of Sciences and Technology, Nara, Japan). It contains yajL disrupted by A red in the Escherichia coli strain BW25113 [lacY citB rbs3 MacZ787 hsdR514 A (araBAD)567 A (rhaBAD)568 rpf1], leading to strain JW5607 (Baba et al., 2006). We transduced the yajL mutation into strain MG1655 as described previously (Messaoudi et al., 2013). For complementation experiments, the yajL mutant was transformed by plasmid pCA24N-yajL as described previously (Le et al., 2012). Cells were grown aerobically in Luria–Bertani (LB) medium at 37 °C.

NAD, NADH, NADP, NADPH, ATP, ADP and AMP pools. Pools of metabolites were measured as described previously (Richarme, 1987), and calculations were performed by assuming 130 μg cell protein (ml bacteria)⁻¹ at A₆₀₀ 1 and 5 μl cell volume (mg protein)⁻¹. The results represent the mean of three experiments (SEM was < 15%).

**NADH dehydrogenase 1 and 2 activities.** NADH dehydrogenases 1 and 2 activities in membrane extracts (prepared from exponential-phase bacteria harvested at OD₆₀₀ 0.4) were assayed by measuring the oxidation of 2,6-dichlorophenolindophenol at 600 nm, with NADH (NADH dehydrogenase 1 + NADH dehydrogenase 2) or deaminomycin (NADH dehydrogenase 1) as substrates (Calhoun & Gennis, 1993). The assay mixture contained the following in a final volume of 1 ml: 0.1 M Tris, pH 7.5, 100 μM NADH or deaminoo-NADH, 30 mM KCN and 10 μg membranes prepared by the French press procedure. An activity of 100 represents 0.18 μmol NADH min⁻¹ (mg protein)⁻¹. The results represent the mean of three experiments.

**NADH, succinate, pyruvate, d-alanine, D-glycerolphosphate and formate oxidase activities.** Oxidases present in membrane extracts were estimated by measuring the rate of oxygen uptake using a Clarke-type oxygen electrode. The reaction mixtures contained membranes (3 mg protein) and substrates (0.6 mM NADH, 20 mM succinate, 4 mM lactate, 20 mM 2-glycerolphosphate) in 100 mM Tris, pH 7.5, 20 mM magnesium acetate and 0.25 M sucrose (Wallace & Young, 1977). Oxygen uptake due to the presence of endogenous substrates was allowed to reach a steady-state level before substrates were added, except for the estimations for succinate oxidase, in which case succinate was added to the reaction mixture before the membranes (Wallace & Young, 1977). The concentration of oxygen dissolved in the reaction buffer at 30 °C was taken to be 225 nmol ml⁻¹. The results represent the mean of three experiments.

**Analysis of culture supernatants.** Bacteria were grown in LB medium (100 μl bacteria in a 1 l Fernbach flask) to exponential phase (OD₆₀₀ 0.8) (both strains displayed exponential growth with identical doubling time until OD₆₀₀ 1.6), and culture supernatants were analysed enzymically for the determination of acetate, lactate, ethanol, succinate and formate. These determinations were made by using the corresponding Megazyme assay kits for acetic acid, lactic acid, ethanol, acetic acid and formic acid, following instructions of the manufacturer (Megazyme). The results represent the mean of three experiments.

**DNA microarray measurements.** The yajL mutant and its parental strain MG1655 were grown under aeration to exponential phase (OD₆₀₀ 0.3) in LB rich medium (Kthiri et al., 2010a). Total RNAs were extracted and treated twice with DNase I (Brumaghim et al., 2003; Collier et al., 2004). RNA quality was monitored with a 2100 Bioanalyzer (Agilent). Transcriptome experiments were performed using E. coli Affymetrix DNA chips by Cogenics according to the standard manufacturer’s instructions. Hybridized arrays were stained using the Affymetrix protocol. Samples were duplicated biologically and we calculated the mean of gene expression ratios from both experiments; genes were considered to be clearly induced if the absolute value of the expression ratio was > 2 and genes displaying too low a signal intensity were discarded from the analysis.

**Microarray analysis and data processing.** After image quantification and global signal correction (following the manufacturer’s instructions), the ratios between intensity values measured in the yajL mutant and its parental strain MG1655 were calculated and normalized using the standard LOWESS (locally weighted scatterplot smoothing) procedure (Messaoudi et al., 2013). This statistical correction has the advantage of removing intensity-dependent effects in the observed log(ratio) values and is thus well adapted to correcting systematic bias related to low-intensity measures. As an additional filter, probes with low-intensity signals in the yajL mutant (< 250) compared with

http://mic.microbiologyresearch.org
background distribution were excluded from subsequent analyses. Reproducibility between results obtained with different microarray replicates was verified and used to eliminate genes with artefactual signals. Finally, to assess whether any of the cellular functions associated with the genes identified as upregulated in the yajL mutant were observed at a frequency greater than that expected by chance, P values were calculated as described previously (Messaoudi et al., 2013) (hypergeometric distribution). Note that this approach was successfully applied previously (Messaoudi et al., 2013). The microarray data were deposited at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/).

Materials. All chemicals were reagent grade and were obtained from Sigma.

RESULTS

Increased NADH/NAD ratio

NADH is produced primarily by catabolic reactions, including glycolysis and the Krebs cycle, and is reoxidized by the respiratory chains and/or fermentations, whereas NADPH, which is produced primarily by the pentose phosphate pathway and isocitrate dehydrogenase, drives anaerobic reactions (Brumaghim et al., 2003; Holm et al., 2010; Lin et al., 2004) and acts as a major reductant during oxidative stress (Holm et al., 2010). We measured the nucleotide pools of aerobic bacteria grown in LB medium during the exponential phase and found that the NADH/NAD ratio of the parental strain (NADH/NAD ratio 0.22) was similar to that reported by others (Auriol et al., 2011), whereas that of the yajL mutant (NADH/NAD ratio 0.72) was similar to ratios observed in NADH dehydrogenase I mutants (Prüss et al., 1994) and anaerobic bacteria (de Graef et al., 1999) (Fig. 1a). We also measured the NADH/NAD ratio of the yajL mutant transformed with the YajL-overproducing plasmid pCA24N-yajL and found that it displayed a ratio of 0.28, close to that of the WT strain (not shown). The high NADH/NAD ratio of the yajL mutant likely results from its NADH dehydrogenase defect (see below) and is probably responsible for respiro-fermentative metabolism described below.

Fig. 1. Nucleotide pools and NADH dehydrogenase activities. (a) NAD (black bars) and NADH (grey bars) pools, and (b) NADP (black bars) and NADPH (grey bars) pools were measured as described in Methods. (c) ATP (black bars), ADP (dark grey bars) and AMP (light grey bars) pools were measured as described in Methods. (d) The activities of NADH dehydrogenases 1 (black) and 2 (grey) in membrane extracts were assayed by measuring the oxidation of 2,6-dichlorophenolindophenol at 600 nm with NADH (NADH dehydrogenase 1 + NADH dehydrogenase 2) or deamino-NADH (NADH dehydrogenase 1) as substrates. An activity of 100 represents 0.18 μmol NADH min⁻¹ (mg protein)⁻¹.
Increased NADPH/NADP ratio

The \textit{yajL} mutant displayed a higher NADPH/NADP ratio (NADPH/NADP ratio 2.0) than the parental strain (NADPH/NADP ratio 1.4) and other WT \textit{E. coli} strains grown under similar conditions (Andersen & von Meyenburg, 1977) (Fig. 1b). This high NADPH/NADP ratio may be important for oxidative stress resistance.

Decreased adenylate energy charge

Intracellular ATP, ADP and AMP concentrations were 1.2 ± 0.16, 0.92 ± 0.12 and 0.42 ± 0.06 mM, respectively, in the \textit{yajL} mutant, versus 2.2 ± 0.28, 0.75 ± 0.09 and 0.21 ± 0.04 mM, respectively, in the parental strain (Fig. 1c). As the mutant displayed higher ADP/ATP and AMP/ATP ratios than the parental strain, its adenylate energy charge (0.58) was lower than that of the parental strain (0.78). This low adenylate energy charge likely favours glycolytic ratios than the parental strain, its adenylate energy charge (NADPH/NADP ratio 1.4) and other WT (NADPH/NADP ratio 2.0) than the parental strain

NADH dehydrogenase defect

\textit{E. coli} possesses two NADH dehydrogenases, i.e. NADH dehydrogenase 1, composed of the \textit{nuoA–N} gene products (a homologue of mitochondrial respiratory complex I), and NADH dehydrogenase 2, the \textit{ndh} gene product. We measured the oxidation of dichlorophenolindophenol with NADH (NADH dehydrogenase 1 + NADH dehydrogenase 2) or deaminoo-NADH (NADH dehydrogenase 1). The WT strain and the \textit{yajL} mutant oxidized NADH at relative rates of 100 and 29, respectively, and deamino-NADH at relative rates of 41 and 5.3, respectively (Fig. 1d); thus NADH dehydrogenase I was almost inactive in the mutant (13 % of the parental strain activity, as reported previously; Le et al., 2012) [we also reported previously that the YajL-overproducing plasmid efficiently rescued (up to 86 %) the NADH dehydrogenase defect of the \textit{yajL} mutant; Le et al., 2012], whereas NADH dehydrogenase 2 was 41 % active. The weak global NADH dehydrogenase activity of the \textit{yajL} mutant may be responsible for the high NADH/NAD ratio, fermentative metabolism and induction of alternative respiratory pathways (see below).

Overexpression of alternative respiratory dehydrogenases

The major respiratory dehydrogenases of \textit{E. coli} are NADH dehydrogenase 1, NADH dehydrogenase 2 and succinate dehydrogenase. Other aerobic dehydrogenases include pyruvate oxidase PoxB, \textit{d}-amino acid dehydrogenase DadA, glycerol 3-phosphate dehydrogenase GldD, formate dehydrogenase FdoGHI, acyl-CoA dehydrogenase FadE and proline oxidase PutA, all of which transfer electrons from substrates to quinones without using NAD/NADH (Fig. 2).

The expression levels of mRNAs coding for respiratory dehydrogenases were determined by DNA array experiments (Fig. 3a). Mutant/parental strain gene expression ratios were between 0.4 and 0.8 for \textit{nuoA–N}, and were 0.75 for \textit{ndh} and 1.3 for \textit{sdhABCD}. By contrast, the \textit{yajL} mutant overexpressed several aerobic alternative dehydrogenases, including pyruvate oxidase PoxB (ninefold), \textit{d}-alanine dehydrogenase DadA (counterpart of eukaryotic \textit{d}-amino acid dehydrogenases; fourfold), glycerophosphate dehydrogenase GldD (prokaryotic counterpart of eukaryotic glycerophosphate dehydrogenase GDP2; 12-fold), formate dehydrogenase FdoGHI (threefold), acyl-CoA dehydrogenase FadE (counterpart of eukaryotic acyl-CoA dehydrogenases; twofold) and proline dehydrogenase PutA (counterpart of eukaryotic proline oxidase; twofold).

Using reverse transcription (RT)-PCR, we measured the expression of pyruvate oxidase and alanine dehydrogenase (which play a major role in respiration in the \textit{yajL} mutant, as described below) and glycerol-phosphate dehydrogenase, and found that expression of pyruvate oxidase and \textit{d}-alanine dehydrogenase was increased by threefold in the mutant, whereas glycerol-phosphate dehydrogenase was not significantly overexpressed (not shown). As expected for aerobically grown cells, anaerobic dehydrogenases, such as glycerophosphate dehydrogenase GldPABC, formate dehydrogenase FdoGHI, and hydrogens A and B, were expressed at low levels in both the \textit{yajL} mutant and the parental strain (not shown); thus, whereas NADH dehydrogenases and succinate dehydrogenase constitute the major dehydrogenases active in WT cells, alternative dehydrogenases are overexpressed in the \textit{yajL} mutant to compensate for NADH dehydrogenase deficiency.

Moderate changes in the expression of quinol oxidases

The major \textit{E. coli} quinol oxidases comprise CybABC, which transfers electrons from quinol to oxygen, and thus performs the functions of mitochondrial complexes 3 and 4, and CydAB, which has a higher affinity for oxygen and is usually expressed in microaerobiosis (Unden, 1998). The genes \textit{cyoA} were expressed at similar levels in both strains, whereas expression of \textit{cydAB} was increased by 1.9-fold in the \textit{yajL} mutant, up to a level approaching that of \textit{cyoA} (Fig. 3b) (the expression of genes \textit{cbdABX} coding for the second \textit{bd}-type oxidase was negligible in both strains; not shown). As expected for aerobic cells, genes coding for anaerobic nitrate, nitrite, DMSO, trimethylamine \textit{N}-oxide reductases and hydrogenase C were not significantly expressed in either of the two strains (Fig. 3b, not shown). However, expression of the \textit{frdABCD} genes coding for fumarate reductase, which transfers electrons from quinols to fumarate and is usually repressed in aerobiosis, was increased by 1.8-fold in the mutant, suggesting that oxaloacetate–succinate interconversion occurs in the oxaloacetate–succinate direction (in accordance with its high NADH/NAD ratio and succinate excretion, as described below). Therefore, in contrast to its impressive overexpression of multiple alternative
dehydrogenases, the yajL mutant expressed quinol oxidase CyoABCD at normal levels, and displayed only a twofold overexpression of microaerobic quinol oxidase CydAB (Noda et al., 2006) and fumarate reductase.

**Increased activities of alternative respiratory chains**

To confirm the increased expression of alternative dehydrogenases in the yajL mutant, we investigated its dehydrogenase activities. We prepared membranes from the mutant and parental strains by the French press procedure, incubated them in an oxygraph cell in the presence of respiratory substrates, and measured oxygen consumptions as a function of time (Fig. 4) (Wallace & Young, 1977). The mutant/WT ratios of NADH and succinate oxidase activities were 0.24 and 0.8, respectively; thus, the yajL mutant displayed low NADH oxidase activity (in accordance with its low NADH dehydrogenase activity) and a succinate oxidase activity slightly lower than that of the parental strain.

Pyruvate oxidase and D-alanine oxidase activities were threefold higher in the yajL mutant than in the parental strain, suggesting that they compensate for the NADH dehydrogenase deficiency. Replacement of pyruvate dehydrogenase by pyruvate oxidase constitutes a major metabolic shift known as overflow metabolism (see below), in which pyruvate is directly converted into acetate in a reaction that is not limited by high NADH levels, bypassing pyruvate dehydrogenase, the Krebs cycle and NADH dehydrogenases (pyruvate oxidase transfers electrons directly from pyruvate to quinones, and acetate is excreted). Glycerol-phosphate dehydrogenase activities were low in both the yajL mutant and the parental strain.

Degradation of D-alanine by D-alanine oxidase produces pyruvate, the pyruvate oxidase substrate, so alanine likely constitutes an important metabolite for the yajL mutant. L-Alanine is also metabolized after racemization by alanine racemase DadX, mRNA levels of which are increased by 10-fold in the yajL mutant (not shown). The increased activities of pyruvate and alanine oxidases in the yajL mutant are consistent with the increased excretion of acetate (see below); thus, the yajL mutant displays increased pyruvate and alanine oxidase activities to overcome NADH dehydrogenase deficiency. Alternative oxidases constitute an energetically favourable alternative to fermentations, because, although PoxB and DadA do not expel protons by themselves (Unden, 1998), a proton motive force is generated during electron transfer through cytochrome oxidases.
Fermentative metabolism

Compared with the parental strain, the yajL mutant expressed fourfold more fermentative lactate dehydrogenase LdhA, threefold more acetaldehyde/alcohol dehydrogenase AdhE and twofold more pyruvate decarboxylase FrsA (which degrades pyruvate to acetaldehyde) (Lee et al., 2011) (Fig. 5a). Accordingly, the yajL mutant excreted (as early as in the exponential phase) higher amounts of acetate (twofold), lactate (eightfold), ethanol (2.6-fold) and succinate (twofold) than the parental strain (Fig. 5b).

Therefore, the yajL mutant exhibited active fermentative metabolism in aerobiosis – a characteristic of cells displaying high NADH/NAD ratios (Berrios-Rivera et al., 2004; Holm et al., 2010). The observed increase in excretion of acetate, succinate, lactate and ethanol was in accordance with overexpression of pyruvate oxidase, fumarate reductase, lactate dehydrogenase and alcohol dehydrogenase, respectively.

Overexpression of glycolytic and pentose phosphate pathway enzymes, and of phosphoenolpyruvate (PEP) carboxykinase and malic enzymes

Bacteria grown in LB medium metabolize both carbohydrates and peptides (Baev et al., 2006; Yohannes et al., 2004). Glycolytic enzymes were overexpressed in the yajL mutant, especially those acting downstream of aldolase (i.e. after convergence of the glycolytic and pentose phosphate pathways), such as triose phosphate isomerase (tpiA gene product; 2.8-fold), glyceraldehyde 3-phosphate dehydrogenase (gap gene product; 2.1-fold), 3-phosphoglycerate kinase (pgk gene product; 2.4-fold), enolase (eno gene product; 1.8-fold) and pyruvate kinase (pyk gene product; 1.7-fold). The expression of pentose phosphate pathway enzymes was also increased, including glucose 6-phosphate dehydrogenase (zwf gene product; 1.8-fold), transaldolase B (aldB gene product; fivefold) and transketolase (tkt gene product; 1.7-fold), suggesting that this pathway constitutes a privileged route for conversion of hexose to triose and is responsible for the high NADPH/NADP ratio of the mutant (Fig. 6a).

PEP carboxykinase and malic enzymes were also overexpressed, and may be involved in converting metabolites derived from aspartate and asparagine (fumarate, malate and oxaloacetate) into PEP and pyruvate, thereby feeding the pyruvate oxidase and fermentative pathways. By contrast, PEP carboxylase (ppc gene product), which catalyses the conversion of PEP into oxaloacetate for replenishment of the Krebs cycle, was underexpressed in...
the yajL mutant (not shown), in accordance with depressed Krebs cycle activity, as discussed below.

These results suggested that metabolism is increased in the yajL mutant through glycolytic and pentose phosphate pathways, in accordance with the observed lower adenylate energy charge, pyruvate-based overflow metabolism, fermentative metabolism and NADPH requirement for combating oxidative stress (Koebmann et al., 2002). The expression levels of PEP carboxykinase (pck gene product) and of a malic enzyme (maeA gene product), which replenish PEP and pyruvate pools from oxaloacetate and malate, were also increased (Fig. 6a).

Overflow metabolism

In aerobic bacteria, the major route for pyruvate degradation is via pyruvate dehydrogenase, the Krebs cycle and respiratory chains. In E. coli, pyruvate is also metabolized into acetate either via the sequential action of pyruvate dehydrogenase, phosphotransacetylase and acetate kinase or via the activity of pyruvate oxidase (Eiteman & Altman, 2006). Pyruvate oxidase was overexpressed by several-fold in the yajL mutant, which suggests that the mutant preferentially uses pyruvate oxidase for acetate production. Moreover, pyruvate dehydrogenase is likely inhibited in the mutant by the high NADH/NAD ratio. In the mutant, pyruvate is also degraded into lactate and ethanol, and the pyruvate–alanine interconversion likely functions in the alanine → pyruvate direction via the alanine dehydrogenase DadA and thus contributes to pyruvate formation. Therefore, overflow metabolism is active in the yajL mutant, with a significant fraction of pyruvate being metabolized into acetate via pyruvate oxidase and into lactate and ethanol by lactate and alcohol dehydrogenases.

Fig. 5. Fermentation. (a) Expression of fermentative enzymes in the yajL mutant (grey bars) and the parental strain (black bars). DH, dehydrogenase. (b) Excretion of acetate, lactate, ethanol, succinate and formate from the yajL mutant (grey bars) and the parental strain (black bars). Bacteria were grown in LB medium at 37 °C and culture supernatants were tested at different times for the presence of fermentation products as described in Methods. A value of ‘1’ represents a concentration of 1 mM in the culture medium for all products, except acetate for which it represents a concentration of 10 mM.

Fig. 6. Expression of intermediary metabolism enzymes and amino acid catabolism. (a) Ratios of mRNA expression values (taken from microarray data) for several metabolic enzymes involved in glycolysis, the pentose phosphate pathway and neoglucogenesis. (b) Amino acid utilization efficiencies (ratio of the yajL mutant/parental strain) by bacteria during exponential growth on casein hydrolysate medium during the second hour after dilution from overnight cultures.
Depressed Krebs cycle

In aerobic WT bacteria, substrates are completely oxidized via the Krebs cycle and the NADH that is produced is oxidized by respiratory chains. Several findings suggest that the Krebs cycle is weakly active in the \textit{yajL} mutant: (i) a high NADH/NAD ratio is known to inhibit pyruvate dehydrogenase, citrate synthase, ketogluatrate and malate dehydrogenases; (ii) NADH dehydrogenases which oxidize Krebs cycle NADH are weakly active; (iii) alternative dehydrogenases utilize Krebs cycle-independent substrates (mainly pyruvate and alanine); (iv) the mutant overexpressed fumarate dehydrogenase and excretes succinate, which suggests that the oxaloacetate/succinate interconversion occurs in the oxaloacetate→succinate direction; and (v) the ketogluatrate pool was 10-fold smaller than that of the parental strain (11 versus 110 μM; not shown), suggesting that ketogluatrate dehydrogenase activity is negligible ($K_{m\text{ketogluatrate}}=140$ μM). Therefore, the Krebs cycle is likely depressed in the \textit{yajL} mutant, and pyruvate-dependent metabolism is characterized by fermentation and pyruvate-related respiration involving d-alanine dehydrogenase and pyruvate oxidase.

Defective amino acid metabolism

As the \textit{yajL} mutant displays reduced Krebs cycle activity and contains low ketogluatrate levels, growth on mixed amino acids is affected, and degradation of pyruvate-forming, transaminase-independent amino acids is favoured, over that of Krebs cycle-related, transaminase-dependent amino acids (amino acid degradation via transaminases requires alpha-ketogluatrate as a substrate). We cultured the \textit{yajL} mutant and the parental strain in tryptone broth (a casein hydrolysate lacking carbohydrates), monitored growth by optical density and removed aliquots of the medium for amino acid analysis at appropriate intervals. The \textit{yajL} mutant grew more slowly than the parental strain in tryptone broth, whereas both strains grew at similar rates in LB medium (which contains sugars and peptides) (not shown), suggesting that it harboured an amino acid utilization defect. Both strains consumed alanine, serine, glycine, aspartate, asparagine and tryptophan rapidly, as reported by others for WT strains (Pruß et al., 1994) (Fig. 6b). The efficient consumption of alanine, serine, glycine and tryptophan by the mutant can be explained by their degradation into pyruvate without transamination. The \textit{yajL} mutant also actively consumed glutamate, glutamine, arginine, phenylalanine, tyrosine, methionine, valine, isoleucine and leucine less efficiently than the parental strain. These amino acids are degraded via Krebs cycle intermediates (oxoglutarate, succinyl-CoA and fumarate) and require a transamination, so their decreased utilization may be explained by the depressed Krebs cycle activity of the mutant and its low ketogluatrate pools. Thus, the \textit{yajL} mutant preferentially degrades pyruvate-forming amino acids that do not require transamination for their degradation.

DISCUSSION

We show in this study that a mutant defective in \textit{YajL}, the proaryotic homologue of the Parkinson’s disease-associated protein DJ-1, displays impressive metabolic changes, including NADH dehydrogenase deficiency, a high NADH/NAD ratio, decreased adenylate energy charge, and pyruvate-centred metabolism involving alternative dehydrogenases and fermentation (Fig. 7).

High NADH/NAD and NADPH/NADP ratios

The high NADH/NAD ratio of the \textit{yajL} mutant is similar to those of NADH dehydrogenase I mutants (Husain et al., 2008) and anaerobic bacteria (de Graef et al., 1999). High NADH/NAD ratios reduce the flow of material through pyruvate dehydrogenase and the Krebs cycle, and favour fermentation and overflow metabolism. The NADPH/NADP ratio in the \textit{yajL} mutant is higher than in the parental strain. NADPH acts as a reductant for peroxides and protein disulfides during oxidative stress, and might be protective against endogenous oxidative stress in the mutant (Husain et al., 2008).

Decreased adenylate energy charge

The low adenylate energy charge of the \textit{yajL} mutant likely triggers high glycolytic flux (Koebmann et al., 2002), as ADP and AMP are allosteric activators of phosphofructokinase and pyruvate kinase. The low adenylate energy charge probably results from fermentative metabolism (Unden, 1998) and alternative respiratory pathways which are less efficient than NADH dehydrogenase in generating a proton motive force (Unden & Bongaerts, 1997). Less tightly coupled respiratory chains may allow the \textit{yajL} mutant to escape to the back pressure from the proton motive force and achieve efficient overflow metabolism (Unden, 1998). Moreover, loosely coupled respiratory chains are advantageous for reducing the formation of reactive oxygen species by respiratory chains. Whereas in bacteria a decrease in the proton motive force occurs by the use of alternative respiratory chains, in eukaryotic cells this is mediated by uncoupling proteins. Interestingly, underexpression of uncoupling proteins 4 and 5 in DJ-1-deficient cells results in defective uncoupling and the overproduction of reactive oxygen species (Guzman et al., 2010).

NADH dehydrogenase 1 deficiency

The NADH dehydrogenase 1 activity of the \textit{yajL} mutant is sevenfold lower than that of the parental strain. As the
NuoG subunit of NADH dehydrogenase 1 is a YajL substrate (Le et al., 2012), the low NADH dehydrogenase 1 activity of the mutant can be explained by its YajL deficiency. The relationship between Parkinson’s disease and complex I inhibition has been studied extensively (Danielson & Andersen, 2008). In the yajL mutant, NADH dehydrogenase 2 is not able to restore a normal NADH/NAD ratio, so the mutant excretes fermentation products and induces NADH-independent alternative dehydrogenases. Human cells do not possess the second NADH dehydrogenase found in bacteria, yeasts and plants, which confers significant flexibility to their respiratory chains. Trans-kingdom gene therapy, however, is a promising strategy for remediing complex I deficiency in humans (Schiff et al., 2012).

**Overexpression of alternative respiratory dehydrogenases**

Alternative dehydrogenases are overexpressed in the yajL mutant and several display increased activity, including pyruvate oxidase PoxB and D-alanine dehydrogenase DadA. In contrast to NADH dehydrogenase 1, which contains many subunits and several FeS clusters, and is sensitive to dysregulated biogenesis and environmental stresses, the alternative dehydrogenases PoxB, GlpD and DadA each consist of a single protein devoid of FeS clusters. The overexpression of alternative dehydrogenases in the mutant could be induced by the stress regulator σS, which is a known inducer of pyruvate oxidase PoxB and alanine dehydrogenase DadA, and is overexpressed in the mutant in the exponential phase (Messaoudi et al., 2013).

Several NADH-independent dehydrogenases described in this study have a eukaryotic counterpart, including succinate dehydrogenase (eukaryotic complex II), glycerophosphate dehydrogenase GlpD (mitochondrial glycerol 3-phosphate dehydrogenase GPD2), D-alanine dehydrogenase DadA (D-amino acid oxidases), acyl-CoA dehydrogenase FadE (fatty acyl-CoA dehydrogenases) and proline oxidase PutA (mitochondrial proline oxidase ProDH). Succinate dehydrogenase likely displays reduced activity in complex
I-deficient eukaryotic cells because of inhibition of the Krebs cycle by the high NADH/NAD ratio. Glycerol 3-phosphate dehydrogenase GDP2, which is part of the NADH shuttle that transfers the reducing power of cytoplasmic NADH to mitochondrial quinones, is interesting because in contrast to the malate/aspartate shuttle, it circumvents mitochondrial NADH production and may bypass NADH dehydrogenase defects. Eukaryotic D-α-amino acid oxidases (which are restricted to D-amino acid detoxification) are of limited metabolic interest. Eukaryotic acyl-CoA dehydrogenases are involved in the first step of fatty acyl-CoA degradation and transfer hydrogens to quinones; however, the next oxidoreduction step of β-oxidation and acetyl-CoA oxidation in the Krebs cycle produce NADH, so they would be of little use to overcome NADH dehydrogenase defects (Abdallah et al., 2007; Bindoff et al., 1989; Calhoun & Gennis, 1993; Koebmann et al., 2002; Le et al., 2012; Schapira & Gegg, 2011). Proline oxidase catalyses the conversion of proline to Δ1-pyrroline-5-carboxylate and transfers hydrogens to quinones via FAD, whereas the reverse reaction, catalysed by Δ1-pyrroline-5-carboxylate reductase, uses NAD(P)H as a cofactor; consequently, both reactions produce a cycle of proline synthesis and degradation that can transfer redox potential between cellular compartments, and might contribute to bypassing NADH dehydrogenase 1 deficiency.

Increase in expression of microaerobic quinol oxidase and fumarate reductase

The yajL mutant overexpresses the microaerobic ubiquinol oxidase CydAB (Unden, 1998) and also fumarate reductase which catalyses the reverse reaction of succinate dehydrogenase, suggesting that the oxaloacetate/succinate branch of the Krebs cycle functions in the reverse direction, as favoured by the high NADH/NAD ratio and in accordance with increased excretion of succinate.

Fermentative metabolism

The yajL mutant displays mixed acid fermentation in accordance with its high NADH/NAD ratio. Human cells utilize lactic fermentations, especially when respiration is unable to cope with high metabolical flux; this occurs when oxygen availability is low during intense exercise or in respiratory chain diseases and cancer. In respiratory chain diseases, fermentation increases as a consequence of the respiratory chain defect, whereas in cancer cells, a high rate of glucose metabolism (aerobic glycolysis, known as the Warburg effect) sustains anabolic reactions (DNA and RNA synthesis), and fermentative metabolism protects cells from intermittent hypoxia.

Amino acid degradation

The yajL mutant favours degradation of pyruvate-forming, transaminase-independent amino acids over that of Krebs cycle-related, transaminase-dependent amino acids. Such a metabolic status might occur in many metabolic diseases, including cancer, mitochondrial respiratory chain diseases and Parkinson’s disease, in which respiratory activities are compromised and/or fermentative metabolism is accelerated. In these cases, adequate amino acid supplementation of patients, considering the two sets of amino acids described above, might be of benefit.

Finally, the present study exemplifies the consequences of a single gene deficiency on cellular metabolism, and constitutes an interesting model for the study of DJ-1 deficiencies and Parkinson’s disease, as well as respiratory chain deficiencies. The remarkable ability of bacteria to cope with NADH dehydrogenase deficiency resulting from YajL deficiency may influence our thinking with regard to the way in which we treat respiratory chain deficiencies in eukaryotic cells by taking into account the differences and similarities between prokaryotic and eukaryotic cells.

ACKNOWLEDGEMENTS

N. M. was supported by a grant from EGIDE.

REFERENCES


