Antonie van Leeuwenhoek
DOI 10.1007/s10482-010-9538-x

PERSPECTIVE

Metabolic networks to combat oxidative stress in *Pseudomonas fluorescens*

Ryan J. Mailloux · Joseph Lemire · Vasu D. Appanna

Received: 12 October 2010 / Accepted: 26 November 2010

Abstract Oxidative stress is an unavoidable peril that aerobic organisms have to confront. Thus, it is not surprising that intricate strategies are deployed in an effort to fend the dangers associated with living in an O_2 environment. In the classical models of anti-oxidative defense mechanisms, a variety of stratagems including the reactive oxygen species (ROS) scavenging systems, the NADPH-generating enzymes and the DNA repair machineries are highlighted. However, it is becoming increasingly clear that metabolism may be intimately involved in anti-oxidative defence. Recent data show that metabolic reprogramming plays a pivotal role in the survival of organisms exposed to oxidative stress. Here, we describe how *Pseudomonas fluorescens*, the metabolically-versatile soil microbe, manipulates its metabolic networks in an effort to counter oxidative stress. An intricate link between metabolism and anti-oxidative defense is presented. *P. fluorescens* reconfigures its metabolic processes in an effort to satisfy its need for NADPH during oxidative insult. Seemingly, disparate metabolic modules appear to partner together to concomitantly fine-tune the levels of the anti-oxidant NADPH and the pro-oxidant NADH. Central to this shift in the metabolic production of the pyridine nucleotides is the increase in NAD kinase with the concomitant decrease in NADP phosphatase. The tricarboxylic acid cycle is tweaked in an effort to limit the formation of NADH. This metabolic redox-balancing act appears to afford a potent tool against oxidative challenge and may be a more widespread ROS-combating tactic than hitherto recognized.

Keywords NADPH–NADH · Homeostasis-antioxidant · Defense-metabolic · Networks-oxidative stress

R. J. Mailloux · J. Lemire · V. D. Appanna (✉)
Department of Chemistry and Biochemistry, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON P3E 2C6, Canada
e-mail: vappanna@laurentian.ca

Present Address:
R. J. Mailloux
Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, 451 Smyth Road, Ottawa, ON K1H 8M5, Canada

Pseudomonas fluorescens is a metabolically versatile organism

Pseudomonas fluorescens is a Gram-negative, rod-shaped and non-pathogenic bacterium that is known to inhabit primarily the soil, plants and water surfaces (Paulsen et al. 2005). It derives its name from its ability to produce fluorescent pigments under iron-limiting conditions (Meyer 2000). This microbe has simple nutritional requirements and can readily thrive in mineral media supplemented with a variety of carbon sources (O'Sullivan and O'Gara 1992). This catabolic versatility coupled with its propensity to

Published online: 12 December 2010

survive in extreme environments has enabled *P. fluorescens* to become an important biotechnological organism. It also affords a unique model system to study metabolic phenomena (Paulsen et al. 2005).

Pseudomonas fluorescens is known to readily modify its metabolism in an effort to thwart the dangers associated with a variety of toxic compounds. The ability of *P.fluorescens* to adapt to a plethora of metal pollutants has been well-documented (Anderson et al. 1992; Cornelis 2008; Hamel and Appanna 2003). While aluminum is primarily processed for elimination as a derivative of oxalate, calcium is essentially sequestered as calcite ($CaCO_3$). Similarly, *P. fluorescens* modifies various metabolic pathways to render gallium innocuous (Beriault et al. 2007). The finding that this microbe generates oxalate to detoxify aluminum, a metal known to induce oxidative stress, indicates that cellular metabolism plays a central role in anti-oxidative defence in *P. fluorescens*. As part of this stratagem to combat oxidative stress, *P. fluorescens* also decreases the formation of nicotinamide adenine dinucleotide hydride (NADH) in order to limit ROS emission from the respiratory chain. The oxidation of NADH via Complex I, Complex III, and Complex IV is a major intracellular generator of ROS (Fig. 1). The manipulation of metabolism in response to an oxidative insult not only results in an increased production of the phosphorylated nicotinamide adenine dinucleotide hydride (NADPH), but also impedes the synthesis of NADH, a response that is pivotal to combating oxidative stress. NADPH is the ultimate reductive force required to neutralize ROS as it

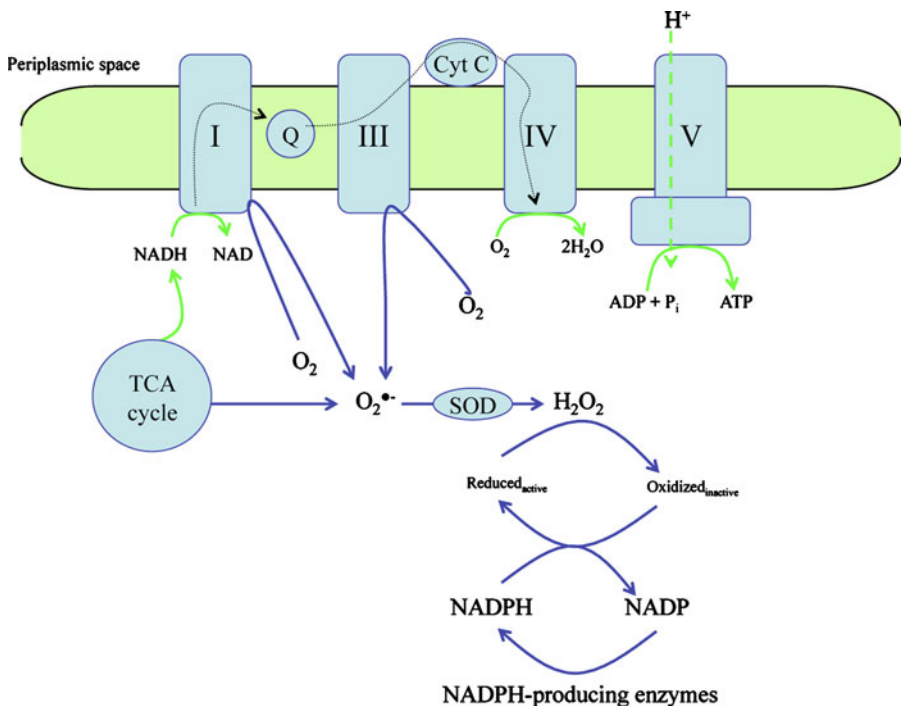

Fig. 1 NADPH is the foundation of anti-oxidative metabolism. The respiratory chain is the main source of ROS in the aerobic cell. The NADH generated by substrate metabolism in the TCA cycle is oxidized by complex I. The liberated electrons are then transferred through a series of prosthetic groups arranged according to increasing redox potential in complexes I, III, and IV to the terminal electron acceptor O_2 (represented by a *dashed arrow*). The favourable transfer of the electrons is coupled to the formation of a proton gradient across the membrane which is tapped by F_oF_1-ATP synthase (complex V) to generate ATP from ADP and P_i. ROS are generated as by-product from this process and are quenched by the anti-oxidative defense systems. Superoxide dismutase (SOD) converts superoxide ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2) which is neutralized by various enzymatic and low molecular weight scavengers. Following oxidative deactivation, H_2O_2 scavengers ($Oxidized_{inactive}$) is reactivated ($Reduced_{active}$) by the reductive power stored in NADPH. NADPH is subsequently regenerated by NADPH-producing enzymes (glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme, NADP-dependent isocitrate dehydrogenase, aldehyde dehydrogenase, NADH kinase, and transhydrogenase)

maintains anti-oxidative systems in reduced/active states (Agledal et al. 2010; Ying 2008). Hence an oxidatively stressed organism will benefit from maintaining low NADH/NAD and high NADPH/NADP redox systems (Aon et al. 2010). For instance, *P.fluorescens* exposed to high amounts of ROS-producing metals manipulate these redox ratios to quench oxidative stress and limit ROS production (Chenier et al. 2008; Lemire et al. 2010a). In this review, we highlight how *P.fluorescens* reconfigures its metabolic networks aimed at favouring NADPH formation and decreasing the production of NADH during oxidative stress.

Aerobic respiration; a risky lifestyle

Aerobic respiration relies on diatomic oxygen (O_2) to drive ATP production. Indeed, by coupling substrate oxidation to the oxidative power of O_2, an aerobic organism can enhance the synthesis of ATP from the chemical energy stored within nutrients. Following substrate oxidation in the tricarboxylic acid (TCA) cycle, electrons from NADH and $FADH_2$ are then passed through respiratory complexes to the terminal electron acceptor O_2 (Fig. 1). The energetically favorable transfer of electrons is coupled to the formation of a proton gradient, which is then tapped by ATP synthase to drive the production of ATP (Dunn and Chandler 1998; Ingledew and Poole 1984). By using O_2 in this manner, aerobic organisms can generate more ATP via substrate oxidation. One caveat is that this process is also accompanied by the formation of ROS (Bergamini et al. 2004; Imlay 2008). Roughly, 0.2–2% of the O_2 consumed by the respiratory chain is univalently reduced to superoxide ($O_2^{\bullet -}$) the chief architect of oxidative stress (Boveris et al. 1980; Cabiscol et al. 2000; Cohen 1994; Droge 2002). The superoxide can then generate hydrogen peroxide (H_2O_2), a far more stable species which can transverse biological membranes (Chance et al. 1979; Hansford et al. 1997). In bacteria, the respiratory chain has been shown to account for up to 87% of the H_2O_2 production in *E.coli* (Gonzalez-Flecha and Demple 1995). These ROS molecules are natural by-products of the respiratory process and under normal conditions partake in cell signaling processes (Forman et al. 2010). However, conditions that inhibit respiration can induce a drastic increase in ROS emission from the respiratory complexes. The major producers of ROS in the respiratory chain include complexes I and III (Hirst et al. 2008; Murphy 2009). In addition, conditions that inhibit electron flow through the complexes can also greatly increase ROS production from the respiratory chain (Iuchi and Weiner 1996; Lemire et al. 2010b). The over-supply of NADH to complex I is well-known to enhance ROS production from the respiratory chain leading to cell death (Murphy 2009). Other enzymes such as α-ketoglutarate dehydrogenase (KGDH), been shown to contribute in ROS production (Adam-Vizi and Chinopoulos 2006; Tretter and Adam-Vizi 2005). The ability of KGDH to generate ROS is sharply increased when NADH is abundant. Hence, NADH is a potent source of intracellular ROS in aerobic organisms.

To control ROS emission from the respiratory chain and avoid oxidative stress, aerobic organisms have developed intricate and redundant strategies to maintain ROS at nontoxic levels (Fig. 1). Superoxide dismutase (SOD) represents the first line of defense against ROS catalyzing the dismutation of $O_2^{\bullet -}$ to H_2O_2 (Winterbourn et al. 1978). The resulting H_2O_2 generated by SOD is then quenched by a suite of anti-oxidative molecules and enzymes, which display a broad range in affinity for H_2O_2. The peroxiredoxin system (PRx) and glutathione/glutathione peroxidase (GSH/GPx) systems are the most efficient enzymatic H_2O_2 quenchers since these systems are located in membrane, periplasmic, and cytosolic environments and their Km values for H_2O_2 are in the μM range (Maddipati and Marnett 1987; Woo et al. 2010). Catalase (CAT) also plays a key part in H_2O_2 detoxification however, this enzyme is only invoked during overt oxidative stress (Havir and McHale 1990). Low molecular weight molecules such as lipoic acid, α-ketoglutarate (KG), and pyruvate, are also well-documented to act as H_2O_2 scavengers (Ambrus et al. 2009; Fedotcheva et al. 2006). Hence, a spectrum of H_2O_2 scavenging systems is used to control the levels of this ROS molecule. The sequestration of H_2O_2 leads to the oxidative deactivation of these enzymes and molecules. To avoid this pitfall, the reductive power stored in NADPH is used to reactivate anti-oxidative defense systems following ROS sequestration (Ying 2008). Specific enzymes such as glutathione reductase (GR) and thioredoxin reductase (TRxR) are required to catalyze the

rejuvenation of the GSH/GR and PRx systems with NADPH. In regard to CAT, NADPH may be bound directly to the enzyme to avoid deactivation by H_2O_2 (Kirkman et al. 1999). Thus, it is essential that NADPH is in constant supply to maintain the anti-oxidative defense system in an active state so that an organism can continue to use O_2 in its aerobic energy quest (Fig. 1).

The pyridine nucleotides NADH and NADPH both play a significant role in the maintenance of cellular ROS homeostasis. While the former produces ROS via the respiratory chain the latter supports anti-oxidative defense. Hence, the manipulation of metabolic pathways and different enzymes involved in pyridine nucleotide metabolism is pivotal to ensure that ROS levels remain in the non-toxic range. Both NADH and NADPH are generated by the enzymatic oxidation of substrates in various metabolic pathways. While NADH is predominantly produced by the TCA cycle, the synthesis of NADPH is essentially mediated by various enzyme-systems. To survive persistent oxidative stress, *P.fluorescens* generates NADPH to counter oxidative stress and maintains low NADH to limit ROS formation from the respiratory chain (Fig. 2) (Singh et al. 2007). During oxidative stress the respiratory chain becomes highly reduced resulting in increased ROS production, a situation that can be exacerbated by increased NADH availability (Adam-Vizi and Chinopoulos 2006). Hence, a metabolic fine-balancing act has to be operative in regard to NADPH and NADH homeostases if an organism is to survive an oxidative environment.

NADPH-producing enzymes and oxidative stress

All aerobic organisms rely on NADPH to counter oxidative stress. The NADPH is supplied by a series of different enzymes located in the TCA cycle, pyruvate shunt, aldehyde metabolism, amino acid metabolism, and pentose phosphate pathway (Pollak et al. 2007a). Indeed, the NADP-dependent isocitrate dehydrogenase (NADP-ICDH), malic enzyme (ME),

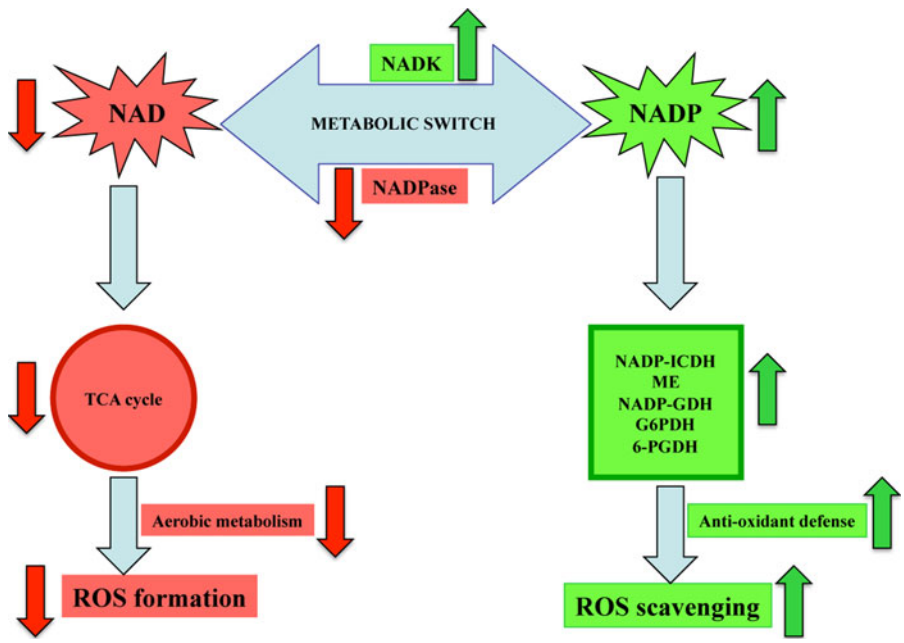

Fig. 2 NADK/NADPase serve as a metabolic switch governing oxidative and anti-oxidative metabolism. In *P. fluorescens* oxidative stress increases NADK and decreases NADPase to preserve the pool of NADP for anti-oxidative defense. Regulation of the synthesis of NAD and NADP culminates in the increased activities of NADPH generating enzymes (NADP-ICDH, G6PDH, ME, 6PGDH, NADP-GDH) and the decreased activity in the TCA cycle. The diminished levels of NADH results in decreased oxidative metabolism and lowers ROS production via the electron transport chain. ↑ = increased activity/expression or abundance of metabolite. ↓ = decreased activity/expression or abundance of metabolite. (Adapted from Singh et al. 2007)

glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6-PGDH), aldehyde dehydrogenase (ADH) and NADP-dependent glutamate dehydrogenase (NADP-GDH) all couple substrate oxidation to the reduction of NADP to NADPH (Singh et al. 2007). The latter is then used to reactivate the anti-oxidant defense system following ROS sequestration. The participation of each of these enzymes in anti-oxidative defense is well-documented (Pollak et al. 2007a; Ying 2008). When challenged by ROS, *P. fluorescens* enhances the production of NADPH in order to quell oxidative stress. Specifically, in-gel activity staining and two-dimensional electrophoresis revealed that NADP-ICDH, G6PDH, ME, 6-PGDH, and NADP-GDH all display a sharp increase in activity and expression in *P. fluorescens* following exposure to menadione, a $O_2^{\bullet -}$ generator, and H_2O_2 (Lemire et al. 2010b; Singh et al. 2007).

Remarkably, *P. fluorescens* expresses three G6PDH isozymes when subjected to oxidative stress, whereas only one of the isozymes is present under control conditions (Singh et al. 2007). The UV-resistant *Deinococcus radiophilus* also invokes several G6PDH isozymes to maintain NADPH levels during oxidative stress (Sung and Lee 2007). NADP-ICDH also appears to be a major contributor to the NADPH pool in *P. fluorescens* (Mailloux et al. 2007). Although NADP-ICDH has been well-studied as a major generator of NADPH in eukaryotes, its role in maintaining this reduced pyridine nucleotide in prokaryotes exposed to oxidative stress is poorly characterized. Based on our findings, it is abundantly clear that NADP-ICDH is a key part of the anti-oxidative arsenal of *P. fluorescens*. Indeed, NADP-ICDH can provide NADPH and KG which are both required for anti-oxidative defense in prokaryotes and eukaryotes alike (Mailloux et al. 2007). Furthermore, *P. fluorescens* elaborates two NADP-ICDH isozymes which are associated with the cytosol (Mailloux et al. 2007). Intriguingly, NADP-ICDH is the major NADPH-producing enzyme in ROS-exposed *P. fluorescens* when citrate is the sole carbon source. Cells grown in malate or histidine use ME and NADP-GDH preferentially to satisfy their NADPH demands (Lemire et al. 2010b; Mailloux et al. 2008). Hence, *P. fluorescens* utilizes a battery of NADPH-producing enzymes to combat oxidative stress. However, it is clear that, depending on the carbon source being used, one NADPH-producing enzyme may be favoured compared to another.

Despite the clear evidence that NADPH is crucial for cell survival, very little information still exists on how organisms select which NADPH-producing enzymes will come to the rescue during oxidative stress. The SOXRS system does account for the induction of G6PDH following an oxidative insult in *Vibrio harveyi* (Vattanaviboon et al. 2003). However, the SOXRS system does not fully account for the dynamic induction of different NADPH-producing enzymes in *P. fluorescens*. One possibility is that the actual carbon source being metabolized may influence the enzyme utilized to generate NADPH. It has recently been demonstrated that the enzymatic source of NADPH varies according to the carbon source being metabolized in *Saccharomyces cerevisiea* (Minard and McAlister-Henn 2005). In this study, when glucose was the sole carbon source both G6PDH and ADH satisfied NADPH demands in the yeast cells. In contrast, yeast cells growing in acetate or oleic acid invoked NADP-ICDH to maintain NADPH levels. Similar observations in mammalian cells were recently reported. When glucose was the primary energy substrate, G6PDH satisfied the requirement for NADPH. In the presence of TCA cycle-linked substrates, the cells relied predominantly on NADP-ICDH (Mailloux and Harper 2010). *P. fluorescens*, apparently, behaves in a similar fashion invoking different NADPH-producing enzymes depending on which carbon source is being utilized. The idea that carbon source regulates NADPH-producing enzyme expression is a novel concept that needs to be further explored. Nonetheless, this hypothesis does account for the dynamic switching between enzymatic sources of NADPH observed in *P. fluorescens* growing on different carbon sources (Fig. 4).

NAD kinase: a metabolic switch to increased NADPH production and decreased NADH synthesis

NAD kinase (NADK) was originally identified by Arthur Kornberg in 1950 and was subsequently discovered as the enzyme required to generate NADP from NAD (Kornberg and Pricer 1950; Magni et al. 1999). NADK is evolutionarily well-conserved and

has been shown to rely on a number of phosphate donors including various inorganic phosphates and nucleotides (ATP, CTP, GTP, and UTP) for the phosphorylation of NAD to NADP (Kawai and Murata 2008; McGuinness and Butler 1985). NADK is known to play a key role in anti-oxidative defense since it is the sole enzyme involved in NADP biosynthesis in all organisms (Grose et al. 2006). Indeed, numerous studies have shown that NADK can substantially increase NADPH biosynthesis and diminish oxidative stress. Knock-down of NADK in HEK-293 cells results in extreme sensitivity to oxidative stress (Pollak et al. 2007b). In contrast, *Arabidopsis thaliana* overexpressing NADK display increased resistance to oxidative stress (Chai et al. 2005).

High NADK has also been shown to aid in the maintenance of elevated NADPH/NADP ratio in metabolically engineered *E.coli* (Lee et al. 2009). In accordance with this, the expression and activity of NADK in *P. fluorescens* markedly increases during the oxidative stress thus enhancing NADP availability for anti-oxidative defense (Singh et al. 2007). Furthermore, HPLC analysis revealed that *P. fluorescens* exposed to ROS readily phosphorylated NAD to NADP (Singh et al. 2007). This response ensures that NADPH is readily available to support anti-oxidative defense. We also found that the NADK in *P. fluorescens* required either ATP or CTP for NADP production which can be supplied by substrate level phosphorylation (Singh et al. 2009; Mailloux et al. 2006). However, whether or not NADK in *P. fluorescens* can use inorganic phosphates for the biosynthesis of NADP has never been tested. In oxidatively stressed *P. fluorescens*, the increase in NADK is matched by decreased NADP phosphatase (NADPase) activity and expression. Indeed, in-gel activity staining revealed that NADPase was decreased in *P. fluorescens* exposed to oxidative stress. NADPase catalyzes the reverse reaction converting NADP into NAD. Unlike NADK, the role of NADPase in the response of various organisms to oxidative stress remains poorly documented. Thus, by modulating NADK/NADPase, *P. fluorescens* ensures that a steady supply of NADP is available for the concomitant formation of NADPH.

As an added benefit, this response also limits NAD availability for NADH formation (Fig. 4). Although *P. fluoresscens* does diminish the activity and expression of various NADH-producing enzymes, limiting NAD ensures that respiratory chain derived ROS will be minimal. Incubation of ROS-exposed *P. fluorescens* in control conditions restored the activity of NADPase and diminished NADK (Singh et al. 2007). Thus, when ROS levels in the cell are high, *P. fluorescens* enhances NADK to favour NADP biosynthesis. Conversely, the return of ROS to normal levels is accompanied by a decrease in NADK and an increase in NADPase to maintain a proper balance of either nucleotide pool. Hence, *P. fluorescens* uses NADK/NADPase as switch to govern the cellular levels of either NADH, a ROS-producing molecule, or NADPH, an anti-oxidant (Fig. 2).

NADH to NADPH conversion cycle

The classical NADPH-generating systems have only limited influence on NADH formation. However, any effective survival stratagem during oxidative insult should couple the enhanced production of NADPH to the diminished synthesis of NADH and a metabolic network is an ideal vehicle to attain this objective. *P. fluorescens* appears to have found the ideal tool to attain this goal. The marshalling of enzymes normally associated with the TCA cycle, glycolysis, and gluconeogenesis to converge into a metabolic network dedicated to the conversion of NADH into NADPH appears to accomplish this task (Singh et al. 2008). Malate dehydrogenase (MDH), malic enzyme (ME), pyruvate carboxylase (PC), and pyruvate kinase (PK) form the hub of this metabolic engine. In addition, the down-regulation of phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate dehydrogenase (PDH) ensures that the necessary substrates are funnelled to this metabolic module.

PEPCK and PC usually work in concert to initiate the synthesis of D-glucose (Liu et al. 2008). However, in *P. fluorescens* challenged by oxidative insult, these two enzymes are uncoupled in order to transform NADH into NADPH (Fig. 3). While the increase in PC converts pyruvate into oxaloacetate, the decrease in PEPCK prevents the passage of oxaloacetate back to phosphoenolpyruvate. Any phosphoenolpyruvate formed is used to produce ATP and pyruvate with the aid of PK. Thus, the uncoupling of PC and PEPCK and the alterations in PK and PDH funnel pyruvate

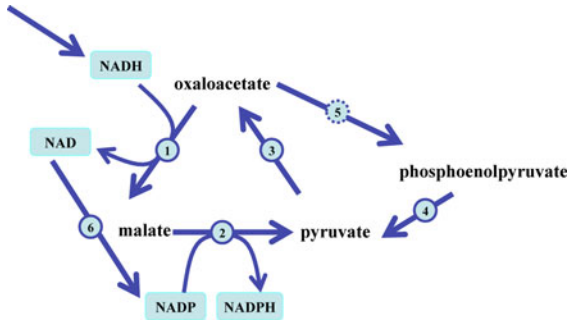

Fig. 3 *P. fluorescens* uses a NADH-to-NADPH conversion cycle to combat oxidative stress. This cycle is generated by the combination of enzymatic elements from the TCA cycle, gluconeogenesis, and glycolysis. *Circles with bold lines* = increase in activity/expression. *Circles with dashed lines* = decrease in activity/expression during oxidative stress. (1) Malate dehydrogenase, (2) malic enzyme, (3) pyruvate carboxylase, (4) pyruvate kinase, (5) phosphoenolpyruvate carboxykinase, (6) NAD kinase

towards oxaloacetate formation, a molecule that acts as a sink for NADH. The malate formed by MDH would be quickly metabolized by ME, a process that generates NADPH and pyruvate. Indeed, blue native gel analysis and in-gel activity staining revealed that the aforementioned enzymes were all increased in activity in the ROS-exposed *P. fluorescens* (Singh et al. 2008). HPLC analysis confirmed that this novel metabolic pathway is operative only in ROS-exposed *P. fluorescens*. Indeed, over a period of 60 min, NADH levels decreased while NADPH levels increased suggesting the conversion of the former into the latter. A concomitant accumulation of pyruvate with the quick reduction of oxaloacetate was also observed during the 60 min period (Fig. 3). In addition, the chemical inhibition of ME with 3-bromopyruvate in the ROS-treated cells resulted in the accumulation of malate confirming this pathway was indeed being used to convert NADH into NADPH (Singh et al. 2008). Thus, ME, MDH, PK and PC appear to work in tandem to convert NADH into NADPH during oxidative stress in *P. fluorescens*.

Metabolic adaptation is at the heart of cellular survival. Alterations in the flux of metabolites can create novel metabolic networks allowing an organism to thrive in a changing environment. Therefore, we propose that the use of metabolism to fend ROS is

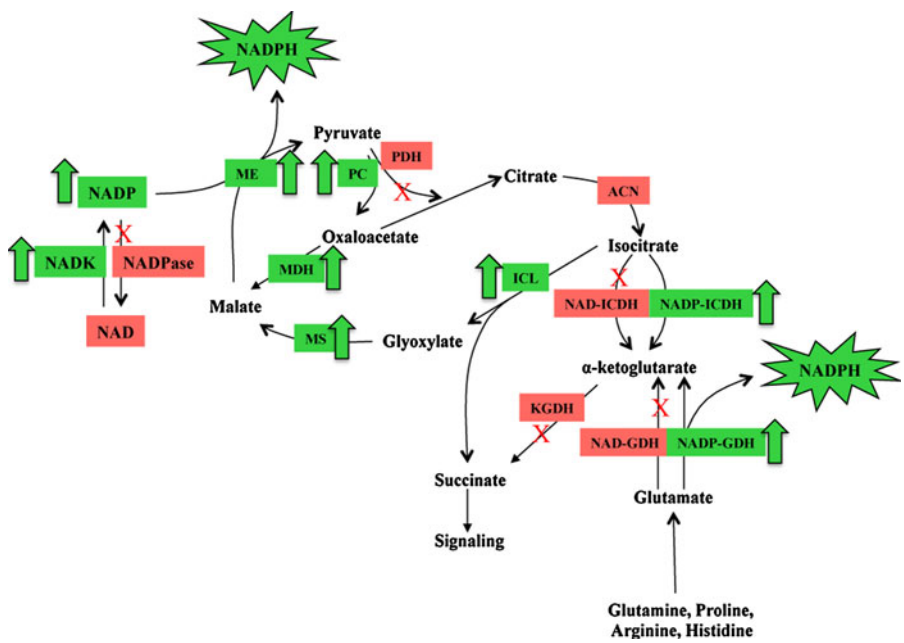

Fig. 4 *P. fluorescens* copes with oxidative stress by tailoring various metabolic pathways to favour NADPH synthesis, the driving force of anti-oxidative defense. The manipulation of these fundamental pathways also results in decreased NADH synthesis, a response required to decrease ROS production from the respiratory chain and KGDH. The NADK switch coupled with the participation of key enzymes involved in "housekeeping" pathways such as the TCA cycle, glycolysis, the glyoxylate cycle, and gluconeogenesis control both NADPH and NADH levels. The upregulation of NADPH networks with concomitant decrease in NADH generation ensures ROS detoxification. *X* = decreased activity/expression. ↑ = increased activity/expression

a universal biological phenomenon in aerobic organisms as it provides a two-pronged approach, i.e. increase in NADPH production and decrease in NADH to nullify oxidative stress. The fluidity of these "housekeeping" metabolic processes and the multifunctional attributes of the metabolites that drive them render this strategy very efficient (Fig. 4).

Concluding remarks

Although NADPH has long been recognized as the main reductive power that enables the survival of all organisms during oxidative stress, its homeostasis has yet to be fully understood. Here we have discussed how certain metabolic networks dedicated to the transformation of NADPH from NADH, provide a remarkable tool to combat oxidative stress in *P. fluorescens*. This molecular stratagem not only increases NADPH formation but concomitantly shifts cellular metabolism away from NADH synthesis, a promoter of ROS (Fig. 4). Such an approach is undoubtedly more effective than the classical one-step enzymatic routes to the genesis of NADPH where the NADH-generating processes may go unabated. We propose that NADK acts as a metabolic switch that controls the levels of NADP and NAD in *P. fluorescens*. In response to an oxidative insult, NADK shifts the cellular metabolism towards processes generating NADPH and limits the activities of NADH-producing networks. We further suggest that the manipulation of metabolic pathways and the elaboration of hitherto unidentified metabolic modules dedicated to the transformation of NADH into NADPH may be two important tools that organisms invoke to combat oxidative stress. This global metabolic strategy may be a universal phenomenon in aerobic organisms and awaits further exploration.

Acknowledgments This work was supported by the Northern Ontario Heritage Fund, Ontario Center of Excellence, and Industry Canada. J. Lemire is a recipient of the Alexander Graham Bell doctoral fellowship.

References

Adam-Vizi V, Chinopoulos C (2006) Bioenergetics and the formation of mitochondrial reactive oxygen species. Trends Pharmacol Sci 27:639–645

Agledal L, Niere M, Ziegler M (2010) The phosphate makes a difference: cellular functions of NADP. Redox Rep 15:2–10

Ambrus A, Tretter L, Adam-Vizi V (2009) Inhibition of the alpha-ketoglutarate dehydrogenase-mediated reactive oxygen species generation by lipoic acid. J Neurochem 109(Suppl 1):222–229

Anderson S, Appanna VD, Huang J, Viswanatha T (1992) A novel role for calcite in calcium homeostasis. FEBS Lett 308:94–96

Aon MA, Cortassa S, O'Rourke B (2010) Redox-optimized ROS balance: a unifying hypothesis. Biochim Biophys Acta 1797:865–877

Bergamini CM, Gambetti S, Dondi A, Cervellati C (2004) Oxygen, reactive oxygen species and tissue damage. Curr Pharm Des 10:1611–1626

Beriault R, Hamel R, Chenier D, Mailloux RJ, Joly H, Appanna VD (2007) The overexpression of NADPH-producing enzymes counters the oxidative stress evoked by gallium, an iron mimetic. Biometals 20:165–176

Boveris A, Sies H, Martino EE, Docampo R, Turrens JF, Stoppani AO (1980) Deficient metabolic utilization of hydrogen peroxide in *Trypanosoma cruzi*. Biochem J 188:643–648

Cabiscol E, Tamarit J, Ros J (2000) Oxidative stress in bacteria and protein damage by reactive oxygen species. Int Microbiol 3:3–8

Chai MF, Chen QJ, An R, Chen YM, Chen J, Wang XC (2005) NADK2, an *Arabidopsis* chloroplastic NAD kinase, plays a vital role in both chlorophyll synthesis and chloroplast protection. Plant Mol Biol 59:553–564

Chance B, Sies H, Boveris A (1979) Hydroperoxide metabolism in mammalian organs. Physiol Rev 59:527–605

Chenier D, Beriault R, Mailloux R, Baquie M, Abramia G, Lemire J, Appanna V (2008) Involvement of fumarase C and NADH oxidase in metabolic adaptation of *Pseudomonas fluorescens* cells evoked by aluminum and gallium toxicity. Appl Environ Microbiol 74:3977–3984

Cohen G (1994) Enzymatic/nonenzymatic sources of oxyradicals and regulation of antioxidant defenses. Ann N Y Acad Sci 738:8–14

Cornelis P (2008) Unexpected interaction of a siderophore with aluminum and its receptor. J Bacteriol 190:6541–6543

Droge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82:47–95

Dunn SD, Chandler J (1998) Characterization of a b2delta complex from *Escherichia coli* ATP synthase. J Biol Chem 273:8646–8651

Fedotcheva NI, Sokolov AP, Kondrashova MN (2006) Nonezymatic formation of succinate in mitochondria under oxidative stress. Free Radic Biol Med 41:56–64

Forman HJ, Maiorino M, Ursini F (2010) Signaling functions of reactive oxygen species. Biochemistry 49:835–842

Gonzalez-Flecha B, Demple B (1995) Metabolic sources of hydrogen peroxide in aerobically growing *Escherichia coli*. J Biol Chem 270:13681–13687

Grose JH, Joss L, Velick SF, Roth JR (2006) Evidence that feedback inhibition of NAD kinase controls responses to oxidative stress. Proc Natl Acad Sci USA 103:7601–7606

Hamel R, Appanna VD (2003) Aluminum detoxification in *Pseudomonas fluorescens* is mediated by oxalate and

phosphatidylethanolamine. Biochim Biophys Acta 1619:70–76

Hansford RG, Hogue BA, Mildaziene V (1997) Dependence of H_2O_2 formation by rat heart mitochondria on substrate availability and donor age. J Bioenerg Biomembr 29:89–95

Havir EA, McHale NA (1990) Purification and characterization of an isozyme of catalase with enhanced-peroxidatic activity from leaves of *Nicotiana sylvestris*. Arch Biochem Biophys 283:491–495

Hirst J, King MS, Pryde KR (2008) The production of reactive oxygen species by complex I. Biochem Soc Trans 36:976–980

Imlay JA (2008) Cellular defenses against superoxide and hydrogen peroxide. Annu Rev Biochem 77:755–776

Ingledew WJ, Poole RK (1984) The respiratory chains of *Escherichia coli*. Microbiol Rev 48:222–271

Iuchi S, Weiner L (1996) Cellular and molecular physiology of *Escherichia coli* in the adaptation to aerobic environments. J Biochem 120:1055–1063

Kawai S, Murata K (2008) Structure and function of NAD kinase and NADP phosphatase: key enzymes that regulate the intracellular balance of NAD(H) and NADP(H). Biosci Biotechnol Biochem 72:919–930

Kirkman HN, Rolfo M, Ferraris AM, Gaetani GF (1999) Mechanisms of protection of catalase by NADPH. Kinetics and stoichiometry. J Biol Chem 274:13908–13914

Kornberg A, Pricer WE Jr (1950) On the structure of triphosphopyridine nucleotide. J Biol Chem 186:557–567

Lee HC, Kim JS, Jang W, Kim SY (2009) Thymidine production by overexpressing NAD + kinase in an *Escherichia coli* recombinant strain. Biotechnol Lett 31:1929–1936

Lemire J, Mailloux R, Auger C, Whalen D, Appanna VD (2010a) *Pseudomonas fluorescens* orchestrates a fine metabolic-balancing act to counter aluminium toxicity. Environ Microbiol 12:1384–1390

Lemire J, Milandu Y, Auger C, Bignucolo A, Appanna VP, Appanna VD (2010b) Histidine is a source of the anti-oxidant, alpha-ketoglutarate, in *Pseudomonas fluorescens* challenged by oxidative stress. FEMS Microbiol Lett 309:170–177

Liu Y, Dentin R, Chen D, Hedrick S, Ravnskjaer K, Schenk S, Milne J, Meyers DJ, Cole P, Yates J 3rd, Olefsky J, Guarente L, Montminy M (2008) A fasting inducible switch modulates gluconeogenesis via activator/coactivator exchange. Nature 456:269–273

Maddipati KR, Marnett LJ (1987) Characterization of the major hydroperoxide-reducing activity of human plasma. Purification and properties of a selenium-dependent glutathione peroxidase. J Biol Chem 262:17398–17403

Magni G, Amici A, Emanuelli M, Raffaelli N, Ruggieri S (1999) Enzymology of NAD + synthesis. Adv Enzymol Relat Areas Mol Biol 73:135–182 xi

Mailloux RJ, Harper ME (2010) Glucose regulates enzymatic sources of mitochondrial NADPH in skeletal muscle cells; a novel role for glucose-6-phosphate dehydrogenase. Faseb J 24:2495–2506

Mailloux RJ, Singh R, Appanna VD (2006) In-gel activity staining of oxidized nicotinamide adenine dinucleotide kinase by blue native polyacrylamide gel electrophoresis. Anal Biochem 359:210–215

Mailloux RJ, Beriault R, Lemire J, Singh R, Chenier DR, Hamel RD, Appanna VD (2007) The tricarboxylic acid cycle, an ancient metabolic network with a novel twist. PLoS One 2:e690

Mailloux RJ, Lemire J, Kalyuzhnyi S, Appanna V (2008) A novel metabolic network leads to enhanced citrate biogenesis in *Pseudomonas fluorescens* exposed to aluminum toxicity. Extremophiles 12:451–459

McGuinness ET, Butler JR (1985) NAD + kinase—a review. Int J Biochem 17:1–11

Meyer JM (2000) Pyoverdines: pigments, siderophores and potential taxonomic markers of fluorescent *Pseudomonas species*. Arch Microbiol 174:135–142

Minard KI, McAlister-Henn L (2005) Sources of NADPH in yeast vary with carbon source. J Biol Chem 280:39890–39896

Murphy MP (2009) How mitochondria produce reactive oxygen species. Biochem J 417:1–13

O'Sullivan DJ, O'Gara F (1992) Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol Rev 56:662–676

Paulsen IT, Press CM, Ravel J, Kobayashi DY, Myers GS, Mavrodi DV, DeBoy RT, Seshadri R, Ren Q, Madupu R, Dodson RJ, Durkin AS, Brinkac LM, Daugherty SC, Sullivan SA, Rosovitz MJ, Gwinn ML, Zhou L, Schneider DJ, Cartinhour SW, Nelson WC, Weidman J, Watkins K, Tran K, Khouri H, Pierson EA, Pierson LS 3rd, Thomashow LS, Loper JE (2005) Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. Nat Biotechnol 23:873–878

Pollak N, Dolle C, Ziegler M (2007a) The power to reduce: pyridine nucleotides–small molecules with a multitude of functions. Biochem J 402:205–218

Pollak N, Niere M, Ziegler M (2007b) NAD kinase levels control the NADPH concentration in human cells. J Biol Chem 282:33562–33571

Singh R, Mailloux RJ, Puiseux-Dao S, Appanna VD (2007) Oxidative stress evokes a metabolic adaptation that favors increased NADPH synthesis and decreased NADH production in *Pseudomonas fluorescens*. J Bacteriol 189:6665–6675

Singh R, Lemire J, Mailloux RJ, Appanna VD (2008) A novel strategy involved in [corrected] anti-oxidative defense: the conversion of NADH into NADPH by a metabolic network. PLoS One 3:e2682

Singh R, Lemire J, Mailloux RJ, Chenier D, Hamel R, Appanna VD (2009) An ATP and oxalate generating variant tricarboxylic acid cycle counters aluminum toxicity in *Pseudomonas fluorescens*. PLoS One 4:e7344

Sung JY, Lee YN (2007) Isoforms of glucose 6-phosphate dehydrogenase in *Deinococcus radiophilus*. J Microbiol 45:318–325

Tretter L, Adam-Vizi V (2005) Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress. Philos Trans R Soc Lond B Biol Sci 360:2335–2345

Vattanaviboon P, Panmanee W, Mongkolsuk S (2003) Induction of peroxide and superoxide protective enzymes and physiological cross-protection against peroxide killing by a superoxide generator in *Vibrio harveyi*. FEMS Microbiol Lett 221:89–95

Winterbourn CC, French JK, Claridge RF (1978) Superoxide dismutase as an inhibitor of reactions of semiquinone radicals. FEBS Lett 94:269–272

Woo HA, Yim SH, Shin DH, Kang D, Yu DY, Rhee SG (2010) Inactivation of peroxiredoxin I by phosphorylation allows localized H(2)O(2) accumulation for cell signaling. Cell 140:517–528

Ying W (2008) NAD +/NADH and NADP +/NADPH in cellular functions and cell death: regulation and biological consequences. Antioxid Redox Signal 10:179–206