MNADK, a novel liver-enriched mitochondrion-localized NAD kinase

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Summary

NADP$^+$ and its reducing equivalent NADPH are essential for counteracting oxidative damage. Mitochondria are the major source of oxidative stress, since the majority of superoxide is generated from the mitochondrial respiratory chain. Because NADP$^+$ cannot pass through the mitochondrial membrane, NADP$^+$ generation within mitochondria is critical. However, only a single human NAD kinase (NADK) has been identified, and it is localized to the cytosol. Therefore, sources of mitochondrial NADP$^+$ and mechanisms for maintaining its redox balance remain largely unknown. Here, we show that the uncharacterized human gene C5ORF33, named MNADK (mouse homologue 1110020G09Rik), encodes a novel mitochondrion-localized NAD kinase. In mice *MNADK* is mostly expressed in the liver, and also abundant in brown fat, heart, muscle and kidney, all being mitochondrion-rich. Indeed, MNADK is localized to mitochondria in Hep G2 cells, a human liver cell line, as demonstrated by fluorescence imaging. Having a conserved NAD kinase domain, a recombinant MNADK showed NAD kinase activity, confirmed by mass spectrometry analysis. Consistent with a role of NADP$^+$ as a coenzyme in anabolic reactions, such as lipid synthesis, *MNADK* is nutritionally regulated in mice. Fasting increased *MNADK* levels in liver and fat, and obesity dramatically reduced its level in fat. *MNADK* expression was suppressed in human liver tumors. Identification of MNADK immediately suggests a model in which NADK and MNADK are responsible for *de novo* synthesis of NADP$^+$ in cytosol and mitochondria, respectively, and therefore provides novel insights into understanding the sources and mechanisms of mitochondrial NADP$^+$ and NADH production in human cells.

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Key words: NAD, NADP, NADPH, NADK, C5ORF33, MNADK

Introduction

Reactive oxygen species (ROS), such as superoxide, is the main cause of cellular oxidative stress, which has been implicated in numerous pathological conditions, including neurodegenerative diseases, atherosclerosis and aging (Griendling et al., 2000; Balaban et al., 2005; Valko et al., 2007). Nicotinamide adenine dinucleotide phosphate (NADP) maintains a pool of its reducing equivalent (NADPH), which is essential to counteracting oxidative damages, by regenerating cellular oxidative defense systems, in addition to being a universal electron donor in detoxification reactions and reductive synthesis, e.g. those for lipid and cholesterol (Pollak et al., 2007a; Agledal et al., 2010).

NAD phosphorylation, the only known reaction to generate NADP$^+$ from NAD, is carried out by NAD kinases, which are conserved from bacteria to humans (Gerdes et al., 2002; Mori et al., 2005; Grose et al., 2006). In eukaryotes, because NADP$^+$ is membrane-impermeable, organelle-specific NADP$^+$ and NADPH production is critical.

Mitochondria are the major source of oxidative stress, because the majority of superoxide is generated from the mitochondrial respiratory chain (Raha and Robinson, 2000). Only a single human NAD kinase (NADK) has been identified, and this NADK is localized to the cytosol (Pollak et al., 2007b). Because NADP$^+$ cannot pass through the mitochondrial membrane, in human cells the source of mitochondrial NADP$^+$ and NADPH and mechanisms for maintaining its redox balance in mitochondria remain largely elusive.

In both yeast (Ghaemmaghami et al., 2003; Bieganowski et al., 2006) and plants (Muto and Miyachi, 1981; Dieter and Marmé, 1984; Berrin et al., 2005; Chai et al., 2005; Turner et al., 2005), 3 compartment-specific NADKs have been identified. In *Saccharomyces cerevisiae*, for example, POS5 is a NADH kinase that is localized to mitochondria (Outten and Culotta, 2003), while the other two are cytosolic (Kawai et al., 2001; Shi et al., 2005). Strikingly, in mammals, only a single NADK has been identified (Lerner et al., 2001). Clearly, the mammalian pathway in *de novo* generation of mitochondrial NADP$^+$ is missing.

Here, we show that the uncharacterized human gene C5ORF33, named MNADK (mouse homologue 1110020G09Rik, Table 1) encodes a novel mitochondrion-localized NAD kinase. We initially identified MNADK as a novel nutritionally regulated gene through performing RNA-seq on liver and fat in mice treated with either fasting or a high-fat diet. MNADK is mostly expressed in liver, and is localized to mitochondria in hep G2 cells, demonstrated by fluorescence imaging. Being evolutionarily conserved, MNADK has a NAD kinase domain. Indeed, a recombinant MNADK has NAD kinase activity that is confirmed by mass spectrometry analysis. The identification of a novel human mitochondrial NAD kinase suggests a model in which NADK and MNADK are responsible for NADP$^+$ production in

Table 1. IDs of MNADK in databases.

	Mouse	Human
Name	MNADK	MNADK
Symbol	1110020G09Rik	C5orf33
Synonyms	4933430B08Rik	NADKD1
Chromosome	Chr15	Chr5
Location	9001009–9040244 bp	36192694–36242258 bp
RefSeq	NM_001085410	NM_001085411
Ensembl	ENSMUSG00000022253	ENSG00000152620
Entrez	68646	133686
Uniprot	Q14BL1	Q4G0N4

cytosol and mitochondria, respectively, leading to novel insights in understanding the sources and mechanisms for mitochondrial $NADP^+$ and NADPH generation in mammalian cells.

Results

MNADK, highly expressed in liver, is localized to mitochondria

Gene expression pattern provides helpful information in revealing functions. We therefore examined the expression pattern of *MNADK* in various mouse tissues. Three male C57BL6 mice were used to dissect 20 tissues, including hypothalamus, cortex, tongue, stomach, small intestine, large intestine, colon, liver, pancreas, heart, blood vessel, kidney, spleen, lung, muscle, urinary bladder, testis and fat. The fat tissues included epididymal fat, inguinal subcutaneous fat and brown fat. We then performed quantitative PCR analysis to examine *MNADK* expression. *MNADK* was highest expressed in the liver (Fig. 1A). The difference between CT values of *MNADK* and *β-actin* was about 3. That is to say, the expression level of *MNADK* is 1/8 of that of *β-actin*, and therefore *MNADK* is highly expressed in the liver. *MNADK* is also abundant in brown fat, heart, kidney and muscle (Fig. 1A). It is noteworthy that all of these tissues are rich in mitochondria.

Indeed, the software iPSORT (Bannai et al., 2002) predicted a mitochondrial targeting peptide in the N-terminal 30 amino acids. We then examined whether MNADK is localized to mitochondria by fluorescent protein imaging. A fusion protein with a green fluorescent protein (GFP) at the C-terminal of MNADK was made by cloning the MNADK open reading frame into a vector encoding GFP. The vector encoding the fusion protein MNADK-GFP was then co-transfected into 3T3 L1 cells with a vector encoding red fluorescent protein (tRFP) fused with truncated BID (BH3 interacting-domain death agonist), tBID, which localizes to mitochondria. Indeed, red signals, which indicated the location of mitochondria, overlapped with green signals, which indicated the location of MNADK (Fig. 1B–D), showing co-localization of MNADK and tBID. Likewise, in Hep G2 cells, a human hepatocyte cell line, we performed co-transfection with vectors encoding MNADK-GFP and fusion protein of tRFP and PDHA1, pyruvate dehydrogenase alpha 1, a mitochondrion-localized protein, and consistent results were obtained (Fig. 1E–G).

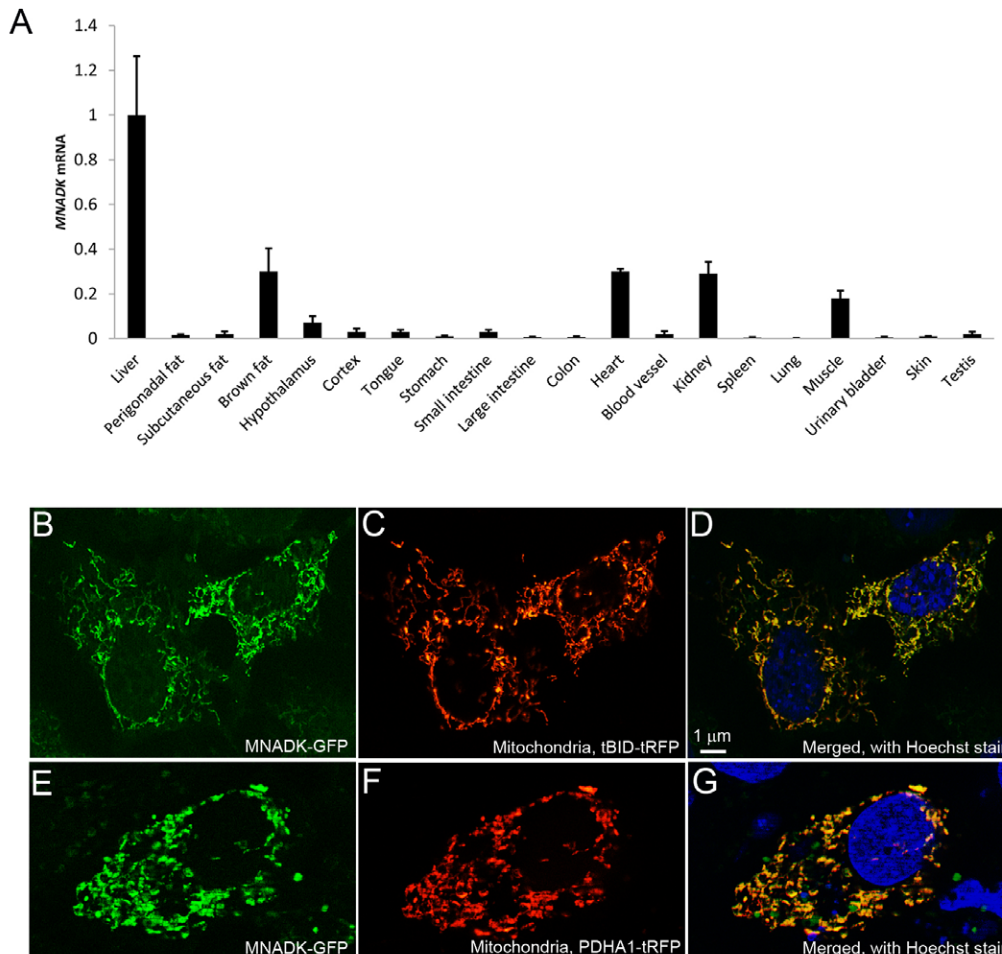

Fig. 1. *MNADK* is highly expressed in liver, and localized to mitochondria. (**A**) In mice *MNADK* is highest expressed in liver, and abundant in brown fat, heart, kidney and muscle, all being mitochondrion-rich. Fluorescence imaging of 3T3 L1 cells transfected with plasmids encoding (**B**) MNADK-GFP, (**C**) tBID-tRFP, a mitochondrial marker, and (**D**) merged pictures. Fluorescence imaging of Hep G2 cells transfected with plasmids encoding (**E**) MNADK-GFP, (**F**) PDHA1-tRFP, a mitochondrial marker, and (**G**) merged pictures. The color blue represents nuclei by Hoechst staining. Scale bar: 1 µm.

Therefore, MNADK is localized to mitochondria, and highly expressed in the liver.

MNADK, highly evolutionarily conserved, has NADH and NAD kinase domains

MNADK is highly evolutionarily conserved. A Blast search using human MNADK protein sequence against the NCBI protein database showed 71 orthologues, including those in mammals, birds, reptiles, fish, fruit fly and even worms, e.g. Y17G7B of *C. elegans*. Between human and mouse proteins, 89% of residues were identical and between human and chicken proteins, 85% of residues were identical (Fig. 2A). Even between protein sequences of human and *C. elegans*, 42% of residues were identical (not shown). Therefore, MNADK is highly conserved. Interestingly, MNADK does not share significant homology with the other human NADK and the yeast NADH kinase POS5.

The software InterProScan (Zdobnov and Apweiler, 2001) assigns an ATP NAD kinase domain (PF01513) to MNADK. Indeed, MNADK has conserved NADH and NAD kinase domains. The human MNADK residues from 111 to 318 showed significant alignment with NADH kinase domain (conserved domain database (Marchler-Bauer et al., 2011), CDD ID, PLN02929; E-value, 7.3×10^{-19}) (Fig. 2B), and residues from 119 to 324 showed significant alignment with conserved NAD kinase domain (CDD ID, COG0061; E-value, 8.5×10^{-13}) (Fig. 2C). Therefore, based on the sequence analysis, it is very likely that MNADK is a NADH and/or NAD kinase.

A recombinant MNADK has NAD kinase activity

To test the hypothesis that MNADK is a NADH and/or NAD kinase, we expressed a recombinant MNADK in *E. coli*. The ORF encoding MNADK isoform 1 was synthesized and cloned into pET 19 expression vector that has a 6-his tag at the N-terminal. The recombinant protein was overexpressed in *E. coli* and purified, resulting in about 80% purity at a concentration of 1 µg/µl, as visualized by Coomassie blue staining (Fig. 3A). The identity of the recombinant protein was further verified by Western blotting using an antibody against MNADK (Fig. 3B). The MNADK protein had a molecular weight of about 49 kDa (Fig. 3A,B).

We then did *in vitro* NAD kinase assay to examine whether the recombinant protein has NAD kinase activity. The recombinant protein was incubated with NAD^+ in a reaction buffer at 37℃ for 10 minutes. $NADP^+$ and NADPH amounts were then measured using a NADP(H) quantification kit. Significant amount of $NADP^+$ was detected in samples containing NAD^+ and MNADK, resulting in an enzyme activity at about 23 units/g, with one unit defined as the amount of enzyme that synthesizes 1 µmol of $NADP^+$ per minute (Fig. 3C). The production of $NADP^+$ was further confirmed by mass spectrometry analysis (Fig. 3D). Incubation of NADH with MNADK also led to generation of NADPH, which, however, was minimal, comparing to that for NAD^+. MNADK is therefore likely a NAD kinase. This conclusion immediately suggests a novel MNADK-based model for $NADP^+$ generation in mitochondria (Fig. 3E).

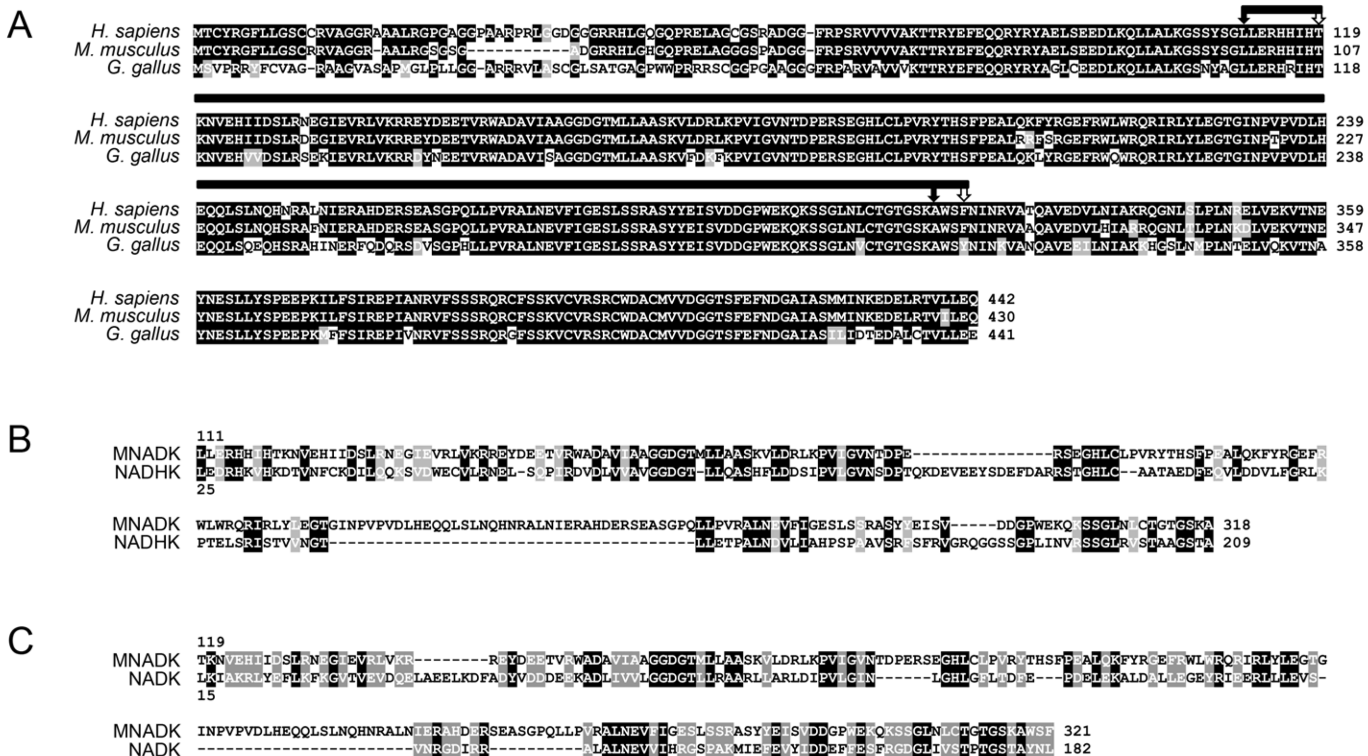

Fig. 2. MNADK is highly evolutionarily conserved, and has NADHK and NADK domains. (**A**) Alignment of MNADK protein sequences of *H. sapiens*, *M. musculus* and *G. Gallus*. The black line denotes NADHK and NADK domains. The segment between the 2 black (open) arrows is homologous to NADH (NAD) kinase domain. (**B**) MNADK has conserved NADH kinase domain. Alignment of MNADK with conserved NADH kinase domain (CDD ID, PLN02929; E-value, 7.3×10^{-19}). (**C**) MNADK has conserved NAD kinase domain. Alignment of MNADK with conserved NAD kinase domain (CDD ID, COG0061; E-value, 8.5×10^{-13}).

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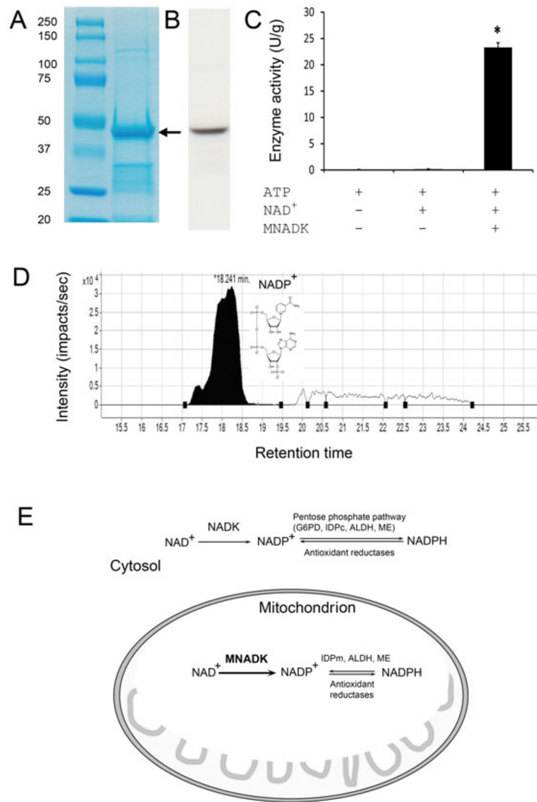

Fig. 3. MNADK has NAD kinase activity. (**A**) Coomassie blue staining for a 6-His tagged recombinant MNADK that is expressed in *E. coli* and (**B**) confirmed by Western blotting using MNADK antibody. (**C**) The recombinant MNADK phosphorylates NAD^+ *in vitro*. One unit is defined as the amount of enzyme that synthesizes 1 µmol of $NADP^+$ per minute. (**D**) $NADP^+$ generation was further confirmed by mass spectrometry analysis. (**E**) A novel model of $NADP^+$ production by MNADK inside the mitochondrion. In this model, NADK and MNADK are responsible for $NADP^+$ production in cytosol and mitochondria, respectively. In cytosol, $NADP^+$ is reduced to NADPH by IDPc, G6PD, ALDH and ME, while in mitochondria this process is through IDPm, ALDH and ME.

MNADK is nutritionally regulated in liver and white adipose tissue and suppressed in human liver tumors

Because $NADP^+$ is a coenzyme used in anabolic reactions, such as lipid synthesis, we hypothesize that MNADK is related to nutritional status. To test this hypothesis, we examined *MNADK* mRNA in liver and fat, including white adipose tissue (WAT) and brown adipose tissue (BAT) in mice treated with a high-fat diet for 3 months or 24-hour fasting.

In the liver, 24-hour fasting increased *MNADK* expression for more than 2 fold ($P<0.01$), and refeeding 4 hours following the fasting normalized its expression (Fig. 4A). No significant difference was observed in mice with HFD treatment (Fig. 4B). In WAT, 24-hour fasting increased *MNADK* expression for about 2.5 fold ($P<0.01$), and refeeding 4 hours following the fasting normalized its expression (Fig. 4C). In mice with 3 months of HFD treatment, *MNADK* expression in WAT was reduced for about 80% ($P<0.01$) (Fig. 3D). *MNADK* expression was not significantly changed in BAT (not shown). Therefore, *MNADK* is nutritionally regulated in liver and WAT.

Mitochondrial functions, such as ROS production, play important roles in tumor progression (Wallace, 2012). Because MNADK is localized to mitochondria and involved in $NADP^+$ generation, we hypothesize that MNADK expression is altered in tumors. To test this hypothesis, we examined *MNADK* expression in normal human livers and human liver tumors at different stages. Indeed, in liver tumors, *MNADK* expression was dramatically reduced, and trended lower with more advanced tumor stages (Fig. 4E).

Discussion

The expression pattern of *MNADK* supports the notion that MNADK critically controls mitochondrial $NADP^+$ production. $NADP^+$ and NADPH play important roles in oxidative defense systems, detoxifying pathways and reductive synthesis, e.g. for lipid and cholesterol, the major functions of the liver, in which *MNADK* is mostly expressed. Consistently, *MNADK* expression levels are nutritionally regulated in liver and fat, and are altered in liver tumors. *MNADK* is also abundant in the heart, muscle, brown fat and kidney, all of which are known to be rich in mitochondria, because mitochondria are needed to produce large amount of energy for mechanical work in heart and muscle (Pagel-Langenickel et al., 2010), for heat production in brown fat (Jacobsson et al., 1985), and for waste excretion in the kidney (García et al., 2012).

It is interesting to compare MNADK and NADK, the only previously known mammalian NAD kinase. MNADK, although highly conserved by itself, does not share significant homology with NADK. NADK is localized in cytosol, while MNADK is localized in mitochondria. We noticed that enzyme activity of MNADK is lower than that of NADK. One possibility is that the recombinant MNADK protein is expressed in *E. coli*, which lacks protein modifications specific to eukaryotic cells, leading to reduced activity. Also it should not be excluded that MNADK, when expressed in eukaryotic cells, can phosphorylate NADH at a comparable activity. Therefore, it is critical to examine MNADK activity *in vivo* to assess its substrate preference and whether it protects cells from ROS challenges.

To combat the constant threat from ROS, cells have evolved numerous anti-oxidant defense systems. NADPH is vital to the regeneration of oxidative defense systems, such as those involving tripeptide glutathione (GSH) and a small redox thioredoxin (TRX), which can serve either as reductants themselves or as cofactors for anti-oxidant enzymes such as GSH peroxidases, glutaredoxins, TRX peroxidases and methionine sulfoxide reductases (Jamieson, 1998; Carmel-Harel and Storz, 2000; Weissbach et al., 2002). NADPH is required for GSH and TRX reductases, which generate reduced GSH and TRX that are needed for anti-oxidant enzymes. In mammalian cells, the pentose phosphate pathway, particularly the rate-limiting enzyme glucose-6-phosphate dehydrogenase (G6PD) located in the cytosol, has been regarded as a critical source of NADPH generation from $NADP^+$ (Pandolfi et al., 1995; Juhnke et al., 1996; Slekar et al., 1996). Other $NADP^+$ reducing enzymes include NADP-specific forms of IDP (isocitrate dehydrogenase), ME (malic enzyme), ALDH (aldehyde dehydrogenase) (Veech et al., 1969; Frenkel, 1971; Bukato et al., 1995; Jo et al., 2001; Koh et al., 2004; Kim et al., 2005).

Mitochondria are the major source of ROS because most ROS is generated through mitochondrial respiratory chain. Therefore, pathways generating $NADP^+$ and NADPH in mitochondria and cytosol are likely different. Mitochondrion-localized NADP dependent isocitrate dehydrogenase (IDPm) and ME isoforms play an important role in NADPH supply in mammals (Frenkel,

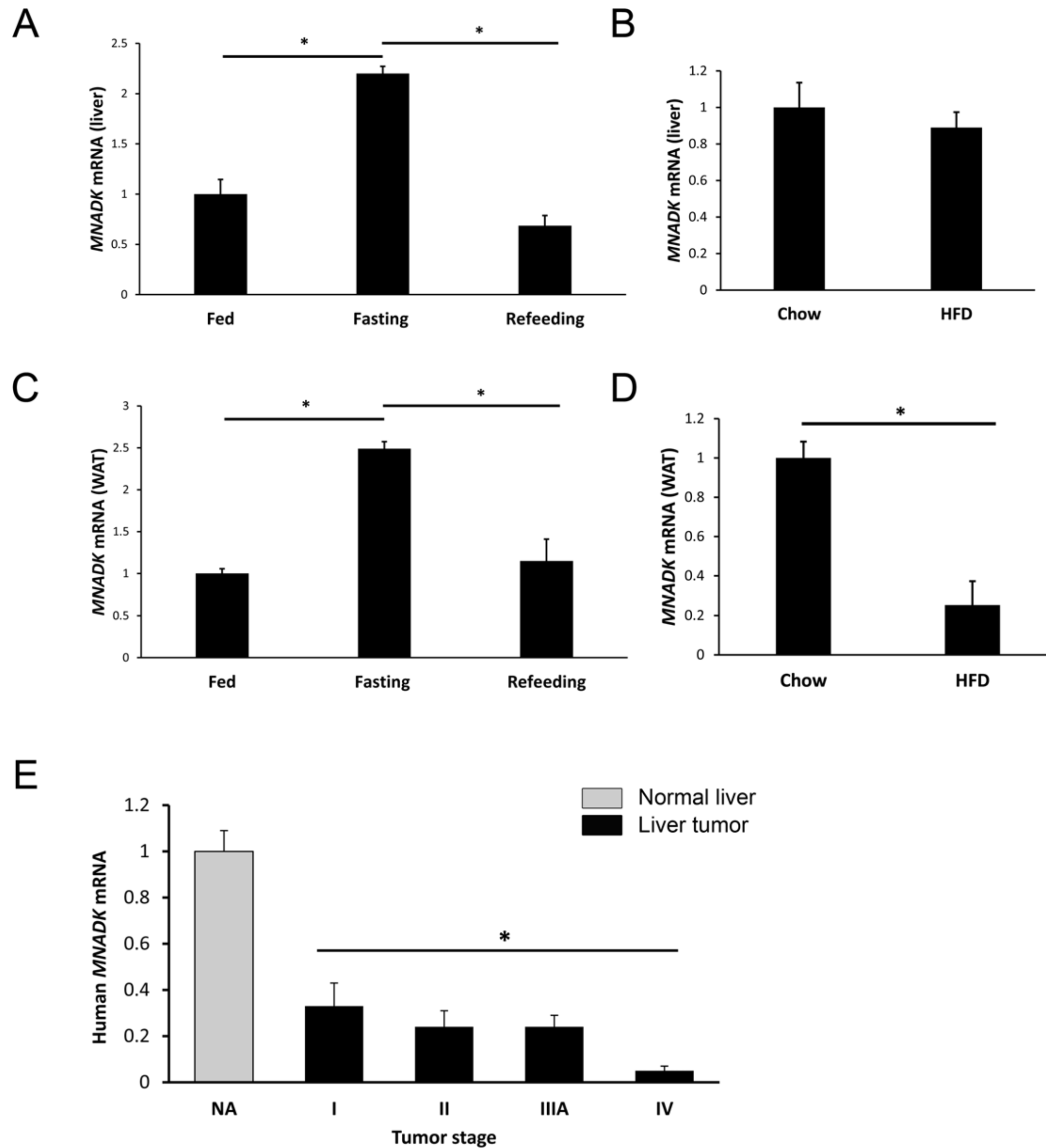

Fig. 4. *MNADK* is nutritionally regulated in liver and white adipose tissue. qPCR analysis for *MNADK* following nutritional stimulation in mice. (**A**) Twenty-four-hour fasting induces liver *MNADK*, which is suppressed 4 hours after refeeding. (**B**) No significant change of liver *MNADK* in mice treated with HFD for 3 months. (**C**) Twenty-four-hour fasting induces WAT *MNADK*, which is suppressed 4 hours after refeeding. (**D**) HFD treatment dramatically suppresses WAT *MNADK*. (**E**) *MNADK* is reduced in human liver tumors. WAT, white adipose tissue; HFD, high fat diet. Data are represented as mean±s.e.m. *$P<0.01$.

1971; Plaut et al., 1983; Bukato et al., 1995; Jo et al., 2001). Identification of a mitochondrial NADK immediately suggests a novel model for compartment-specific generation of $NADP^+$ and NADPH. In this model, NADK and MNADK are responsible for $NADP^+$ production in cytosol and mitochondria, respectively. In cytosol, $NADP^+$ is reduced to NADPH by IDPc, G6PD, ALDH and ME, while in mitochondria, this process is through IDPm, ALDH and ME (Fig. 3E).

In summary, MNADK, an evolutionarily conserved protein, has a conserved NAD kinase domain. MNADK is highly expressed in liver, brown fat, heart, muscle and kidney, all being mitochondrion-rich. We demonstrate that MNADK is localized to mitochondria by fluorescence imaging in cells, and that a recombinant MNADK has NAD kinase activity. The identification of a novel mammalian pathway for *de novo* synthesis of mitochondrial $NADP^+$ will benefit researches on anti-oxidative defenses, detoxification and reductive synthesis.

Materials and Methods

Mice

Mice were housed at 22–24℃ with a 14-hour light, 10-hour dark cycle and provided with *ad libitum* water and a chow diet (6% calories from fat, 8664; Harlan Teklad, Indianapolis, IN) unless otherwise indicated. To examine nutritional stimulation induced *MNADK* expression, 10 4-week-old male C57B6 mice (Jackson laboratory, Bar Habor, ME) were placed on either a chow diet or a high-fat, high-sucrose diet (58% kcal from fat, 26% kcal from sucrose, D-12331; Research Diets, New Brunswick, NJ) for 3 months. Five 8-week-old mice were treated with 24-hour fasting with 4 fed mice as controls. To examine the expression pattern of *MNADK* in various mouse tissues, 3 8-week-old mice were used. All animal protocols were approved by the Animal Care and Use Committee of Wayne State University.

RNA extraction, quantitative real-time PCR and Western blotting analysis

Dissected tissues were immediately placed into RNAlater solution (Ambion, Austin, TX) for subsequent RNA extraction. Total RNA was isolated from tissues with RNeasy tissue minikit with deoxyribonuclease treatment (QIAGEN, Valencia, CA). One microgram of RNA was reverse transcribed to cDNA using random hexamers (Superscript; Ambion). Relative expression levels were calculated and *β-actin* was used as an internal control. Liver tumor cDNA Array (Origene, Rockville, MD) was used to examine *MNADK* expression in human liver tumors, and the array contained normalized cDNA from 8, 7, 8, 8 and 3 individuals with normal liver, liver tumors at stages I, II, IIIA and IV, respectively. Primer sequences for mouse *MNADK* were: forward, 5′-TGCCTGTGCGATACACGCAT-3′; reverse, 5′-TGTCCACTGCTACAGCCCAACA-3′. Primer sequences for mouse *β-actin* were: forward, 5′-GTGACGTTGACATCCGTAAAGA-3′; reverse, 5′-GCCGGACTCATCGTACTCC-3′. Primer sequences for human *MNADK* were: forward, 5′-CTGCTGGCAGCGAGTAAAGT-3′; reverse, 5′-TGTGTATATCGAACGGGCAGG-3′. A polyclonal antibody against C5ORF33 (Abgent, San Diego, CA) was used for doing Western blotting analysis. Qproteome Mitochondria Isolation Kit (QIAGEN, Valencia, CA) was used to isolate mitochondria from either Hep G2 cells or from mouse livers.

Multiple alignments

IDs for MNADK protein sequences from humans, mice and chicken were NP_001078880.1, NP_001035485.2 and XP_425010.3, respectively. The software ClustalW2 (Larkin et al., 2007) was used to perform multiple alignments, and Blast (Altschul et al., 1997) was used to perform pairwise comparison between two sequences. Jalview (Waterhouse et al., 2009) was used to visualize the alignment result.

Expression and purification of recombinant MNADK and measurement of its activity

The DNA sequence for human MNADK (NM_001085411.1) was synthesized (Genscript, NJ) and cloned into pET 19 (Novagen) with an N-terminal 6-His tag. The purified protein was resolved by 10% SDS-PAGE, and then visualized by Coomassie Blue staining. To perform *in vitro* NAD kinase assay (Griffiths and Bernofsky, 1972; Jacobson and Jacobson, 1976; Iwahashi et al., 1989), briefly, 10 µg of recombinant MNADK protein was added into a reaction mixture containing 100 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 10 mM ATP, 5 mM either NAD^+ or NADH, in a final volume of 100 µl, in triplicates, followed by incubation at 37℃ for 10 minutes. The reaction containing NADH or NAD^+ was terminated by alkalization or acidification, respectively, followed by neutralization. The amounts of $NADP^+$ and NADPH were then determined by NADP/NADPH Quantitation Kit according to manufactory instruction (Biovision, CA). The amount of $NADP^+$ was further confirmed by mass spectrometry analysis at core facility of University of Michigan.

Imaging

Fusion protein of MNADK-GFP was made by cloning MNADK open reading frame into the pCMV6-AC-GFP vector. In 3T3 L1 and Hep G2 cells, the MNADK-GFP vector was co-transfected with a pCMV6 vector that encodes a fusion protein of tRFP with either tBID or PDHA1 (Origene, MD). One microgram of plasmids was transfected into the cells that reached about 70% confluence. Twenty-four hours after transfection, cells were fixed with 4% formaldehyde in PBS for 20 minutes followed by washing by cold PBS. Fluorescence Images (40× magnification) were taken at the imaging core facility of Wayne State University with a Leica TCS SP5 Confocal Microscope.

Statistical analysis

Data are expressed as the mean±s.e.m. Statistical significance was tested with unpaired two-tailed Student's *t*-tests. The differences were considered statistically significant if $P<0.05$.

Note added in proof

While the manuscript was being reviewed, we noticed a recent publication showing that C5ORF33 has NAD kinase activity both in yeast and *in vitro* (Ohashi et al., 2012).

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Competing Interests

The author has no competing interests to declare.

References

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

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389-3402.

Balaban, R. S., Nemoto, S. and Finkel, T. (2005). Mitochondria, oxidants, and aging. *Cell* **120**, 483-495.

Bannai, H., Tamada, Y., Maruyama, O., Nakai, K. and Miyano, S. (2002). Extensive feature detection of N-terminal protein sorting signals. *Bioinformatics* **18**, 298-305.

Berrin, J. G., Pierrugues, O., Brutesco, C., Alonso, B., Montillet, J. L., Roby, D. and Kazmaier, M. (2005). Stress induces the expression of *AtNADK-1*, a gene encoding a NAD(H) kinase in *Arabidopsis thaliana*. *Mol. Genet. Genomics* **273**, 10-19.

Bieganowski, P., Seidle, H. F., Wojcik, M. and Brenner, C. (2006). Synthetic lethal and biochemical analyses of NAD and NADH kinases in *Saccharomyces cerevisiae* establish separation of cellular functions. *J. Biol. Chem.* **281**, 22439-22445.

Bukato, G., Kochan, Z. and Swierczyński, J. (1995). Purification and properties of cytosolic and mitochondrial malic enzyme isolated from human brain. *Int. J. Biochem. Cell Biol.* **27**, 47-54.

Carmel-Harel, O. and Storz, G. (2000). Roles of the glutathione- and thioredoxin-dependent reduction systems in the *Escherichia coli* and *Saccharomyces cerevisiae* responses to oxidative stress. *Annu. Rev. Microbiol.* **54**, 439-461.

Chai, M. F., Chen, Q. J., An, R., Chen, Y. M., Chen, J. and Wang, X. C. (2005). NADK2, an *Arabidopsis* chloroplastic NAD kinase, plays a vital role in both chlorophyll synthesis and chloroplast protection. *Plant Mol. Biol.* **59**, 553-564.

Dieter, P. and Marmé, D. (1984). A Ca^{2+}, calmodulin-dependent NAD kinase from corn is located in the outer mitochondrial membrane. *J. Biol. Chem.* **259**, 184-189.

Frenkel, R. (1971). Bovine heart malic enzyme. I. Isolation and partial purification of a cytoplasmic and a mitochondrial enzyme. *J. Biol. Chem.* **246**, 3069-3074.

García, I. M., Altamirano, L., Mazzei, L. J., Fornés, M. W., Molina, M. N., Ferder, L. and Manucha, W. (2012). Role of mitochondria in paricalcitol-mediated cytoprotection during obstructive nephropathy. *Am. J. Physiol.* **302**, F1595-F1605.

Gerdes, S. Y., Scholle, M. D., D'Souza, M., Bernal, A., Baev, M. V., Farrell, M., Kurnasov, O. V., Daugherty, M. D., Mseeh, F., Polanuyer, B. M. et al. (2002). From genetic footprinting to antimicrobial drug targets: examples in cofactor biosynthetic pathways. *J. Bacteriol.* **184**, 4555-4572.

Ghaemmaghami, S., Huh, W. K., Bower, K., Howson, R. W., Belle, A., Dephoure, N., O'Shea, E. K. and Weissman, J. S. (2003). Global analysis of protein expression in yeast. *Nature* **425**, 737-741.

Griendling, K. K., Sorescu, D. and Ushio-Fukai, M. (2000). NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ. Res.* **86**, 494-501.

Griffiths, M. M. and Bernofsky, C. (1972). Purification and properties of reduced diphosphopyridine nucleotide kinase from yeast mitochondria. *J. Biol. Chem.* **247**, 1473-1478.

Grose, J. H., Joss, L., Velick, S. F. and Roth, J. R. (2006). Evidence that feedback inhibition of NAD kinase controls responses to oxidative stress. *Proc. Natl. Acad. Sci. USA* **103**, 7601-7606.

Iwahashi, Y., Hitoshio, A., Tajima, N. and Nakamura, T. (1989). Characterization of NADH kinase from *Saccharomyces cerevisiae*. *J. Biochem.* **105**, 588-593.

Jacobson, E. L. and Jacobson, M. K. (1976). Pyridine nucleotide levels as a function of growth in normal and transformed 3T3 cells. *Arch. Biochem. Biophys.* **175**, 627-634.

Jacobsson, A., Stadler, U., Glotzer, M. A. and Kozak, L. P. (1985). Mitochondrial uncoupling protein from mouse brown fat. Molecular cloning, genetic mapping, and mRNA expression. *J. Biol. Chem.* **260**, 16250-16254.

Jamieson, D. J. (1998). Oxidative stress responses of the yeast *Saccharomyces cerevisiae*. *Yeast* **14**, 1511-1527.

Jo, S. H., Son, M. K., Koh, H. J., Lee, S. M., Song, I. H., Kim, Y. O., Lee, Y. S., Jeong, K. S., Kim, W. B., Park, J. W. et al. (2001). Control of mitochondrial redox balance and cellular defense against oxidative damage by mitochondrial $NADP^+$-dependent isocitrate dehydrogenase. *J. Biol. Chem.* **276**, 16168-16176.

Juhnke, H., Krems, B., Kötter, P. and Entian, K. D. (1996). Mutants that show increased sensitivity to hydrogen peroxide reveal an important role for the pentose phosphate pathway in protection of yeast against oxidative stress. *Mol. Gen. Genet.* **252**, 456-464.

Kawai, S., Suzuki, S., Mori, S. and Murata, K. (2001). Molecular cloning and identification of *UTR1* of a yeast *Saccharomyces cerevisiae* as a gene encoding an NAD kinase. *FEMS Microbiol. Lett.* **200**, 181-184.

Kim, H. J., Kang, B. S. and Park, J. W. (2005). Cellular defense against heat shock-induced oxidative damage by mitochondrial $NADP^+$-dependent isocitrate dehydrogenase. *Free Radic. Res.* **39**, 441-448.

Koh, H. J., Lee, S. M., Son, B. G., Lee, S. H., Ryoo, Z. Y., Chang, K. T., Park, J. W., Park, D. C., Song, B. J., Veech, R. L. et al. (2004). Cytosolic $NADP^+$-dependent isocitrate dehydrogenase plays a key role in lipid metabolism. *J. Biol. Chem.* **279**, 39968-39974.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R. et al. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947-2948.

Lerner, F., Niere, M., Ludwig, A. and Ziegler, M. (2001). Structural and functional characterization of human NAD kinase. *Biochem. Biophys. Res. Commun.* **288**, 69-74.

Marchler-Bauer, A., Lu, S., Anderson, J. B., Chitsaz, F., Derbyshire, M. K., DeWeese-Scott, C., Fong, J. H., Geer, L. Y., Geer, R. C., Gonzales, N. R. et al. (2011). CDD: a Conserved Domain Database for the functional annotation of proteins. *Nucleic Acids Res.* **39**, D225-D229.

Mori, S., Kawai, S., Shi, F., Mikami, B. and Murata, K. (2005). Molecular conversion of NAD kinase to NADH kinase through single amino acid residue substitution. *J. Biol. Chem.* **280**, 24104-24112.

Muto, S. and Miyachi, S. (1981). Light-induced conversion of nicotinamide adenine dinucleotide to nicotinamide adenine dinucleotide phosphate in higher plant leaves. *Plant Physiol.* **68**, 324-328.

Ohashi, K., Kawai, S. and Murata, K. (2012). Identification and characterization of a human mitochondrial NAD kinase. *Nat. Commun.* **3**, 1248.

Outten, C. E. and Culotta, V. C. (2003). A novel NADH kinase is the mitochondrial source of NADPH in *Saccharomyces cerevisiae*. *EMBO J.* **22**, 2015-2024.

Pagel-Langenickel, I., Bao, J., Pang, L. and Sack, M. N. (2010). The role of mitochondria in the pathophysiology of skeletal muscle insulin resistance. *Endocr. Rev.* **31**, 25-51.

Pandolfi, P. P., Sonati, F., Rivi, R., Mason, P., Grosveld, F. and Luzzatto, L. (1995). Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress. *EMBO J.* **14**, 5209-5215.

Plaut, G. W., Cook, M. and Aogaichi, T. (1983). The subcellular location of isozymes of NADP-isocitrate dehydrogenase in tissues from pig, ox and rat. *Biochim. Biophys. Acta* **760**, 300-308.

Pollak, N., Dölle, C. and 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. and Ziegler, M. (2007b). NAD kinase levels control the NADPH concentration in human cells. *J. Biol. Chem.* **282**, 33562-33571.

Raha, S. and Robinson, B. H. (2000). Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem. Sci.* **25**, 502-508.

Shi, F., Kawai, S., Mori, S., Kono, E. and Murata, K. (2005). Identification of ATP-NADH kinase isozymes and their contribution to supply of NADP(H) in *Saccharomyces cerevisiae*. *FEBS J.* **272**, 3337-3349.

Slekar, K. H., Kosman, D. J. and Culotta, V. C. (1996). The yeast copper/zinc superoxide dismutase and the pentose phosphate pathway play overlapping roles in oxidative stress protection. *J. Biol. Chem.* **271**, 28831-28836.

Turner, W. L., Waller, J. C. and Snedden, W. A. (2005). Identification, molecular cloning and functional characterization of a novel NADH kinase from *Arabidopsis thaliana* (thale cress). *Biochem. J.* **385**, 217-223.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* **39**, 44-84.

Veech, R. L., Eggleston, L. V. and Krebs, H. A. (1969). The redox state of free nicotinamide–adenine dinucleotide phosphate in the cytoplasm of rat liver. *Biochem. J.* **115**, 609-619.

Wallace, D. C. (2012). Mitochondria and cancer. *Nat. Rev. Cancer* **12**, 685-698.

Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M. and Barton, G. J. (2009). Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**, 1189-1191.

Weissbach, H., Etienne, F., Hoshi, T., Heinemann, S. H., Lowther, W. T., Matthews, B., St John, G., Nathan, C. and Brot, N. (2002). Peptide methionine sulfoxide reductase: structure, mechanism of action, and biological function. *Arch. Biochem. Biophys.* **397**, 172-178.

Zdobnov, E. M. and Apweiler, R. (2001). InterProScan – an integration platform for the signature-recognition methods in InterPro. *Bioinformatics* **17**, 847-848.

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