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De novo NAD$^+$ synthesis is ineffective for NAD$^+$ supply in axenically cultured *Caenorhabditis elegans*

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To secure an adequate nicotinamide adenine dinucleotide (NAD$^+$) supply for survival, organisms typically rely on two complementary mechanisms: the de novo synthesis pathway and the salvage pathway. Notably, the classic quinolinic acid phosphoribosyltransferase (QPRTase) for de novo NAD$^+$ synthesis is absent in *Caenorhabditis elegans* (*C. elegans*), despite the reported alternative mechanism involving uridine monophosphate phosphoribosyltransferase (UMPS). However, the effectiveness of this proposed mechanism for NAD$^+$ production of *C. elegans* remains unclear. Here, using a chemically defined medium, we observed that removing NAD$^+$ salvage precursors from the medium results in a significant decrease in NAD$^+$ levels, causing severe developmental delay and fecundity loss in *C. elegans*. Strikingly, these defects cannot be restored by any metabolites from the de novo synthesis pathway, including the direct QPRTase substrate quinolinic acid (QA). Furthermore, the deficiency of *umps-1* does not cause any significant changes in the NAD$^+$ levels of *C. elegans*. Moreover, the growth defects of the *umps-1* mutant could be rescued by uridine, but not the salvage NAD$^+$ supply. Additionally, we discovered that commercially available QA products contain substantial amounts of nicotinic acid, potentially producing misleading information. Collectively, our results demonstrate that *C. elegans* lacks the necessary mechanisms for de novo synthesis of NAD$^+$.

NAD$^+$ is required for various biological processes and is thus indispensable for all living cells. As an electron carrier, NAD$^+$ plays a central role in numerous metabolic reactions, such as glycolysis, oxidative decarboxylation, the TCA cycle, fatty acid β-oxidation and alcohol metabolism[1]. It can be phosphorylated to NADP$^+$ by NAD$^+$ kinases and contribute to anabolic reactions. NAD$^+$ also functions as a substrate for NAD$^+$-consuming enzymes (generally called NAD$^+$ consumers), including sirtuins, poly ADP-ribose polymerases (PARPs), CD38, and sterile alpha and TIR motif-containing 1 (SARM1)[2].

In organisms, NAD$^+$ is produced through two main metabolic pathways: the de novo and salvage synthesis pathways (illustrated in the "Results" section). Three forms of vitamin B3, namely, nicotinamide (NAM), nicotinic acid (NA), and nicotinamide riboside (NR), can be converted to NAD$^+$ through the salvage synthesis pathway[3]. Tryptophan, an essential amino acid for organisms, is the primary precursor of de novo NAD$^+$ synthesis. In mammalian cells, tryptophan can be converted to quinolinic acid (QA) via the kynurenine pathway, then to nicotinic acid mononucleotide (NaMN) by quinolinic acid phosphoribosyltransferase (QPRTase), and eventually to NAD$^+$[4]. NAD$^+$ deficiency occurs only when both NAD$^+$ biosynthetic pathways are impaired in mammals, as the NAD$^+$ demand can be effectively complemented by either pathway[5–7]. By sequence homology comparison, the ortholog of QPRTase for de novo NAD$^+$ synthesis was found to be missing in *C. elegans*[8]. However, interestingly, a previous study proposed that the uridine monophosphate synthetase (UMPS) of this worm can maintain de novo NAD$^+$ synthesis in the absence of QPRTase, suggesting the existence of a potential de novo NAD$^+$ synthesis pathway in *C. elegans*[9]. Soon afterward, another study reported that supplementation with tryptophan could increase NAD$^+$ levels via the de novo synthesis pathway in *C. elegans* fed with bacteria[4]. However, whether UMPS can truly replace QPRTase and how efficiently this alternative de novo synthesis pathway can complement the NAD$^+$ demand in *C. elegans* are uncertain.

In the laboratory, *C. elegans* is routinely fed with the bacterium *E. coli*. This culture system is convenient for worm maintenance and genetic

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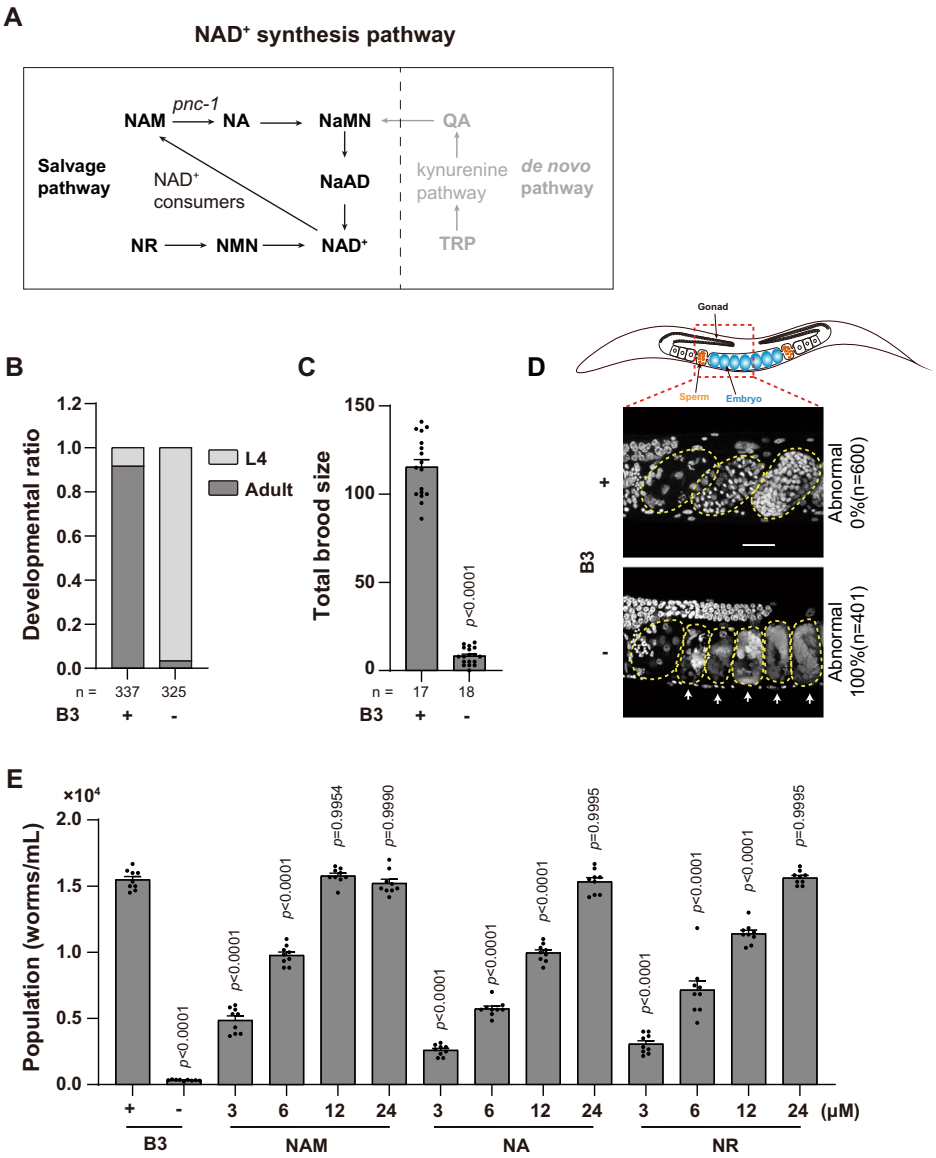

Fig. 1 | The NAD⁺ supply from salvage synthesis is indispensable for the development and reproduction of *C. elegans*. **A** Schematic diagram of NAD⁺ synthesis in *C. elegans*; NMN is nicotinamide mononucleotide. **B** and **C** Physiological examinations of worms cultured in complete or vitamin B3-deficient medium; development (**B**), $N = 3$ independent experiments containing over 300 worms in total; brood size (**C**), $N = 3$ independent experiments containing over 17 worms in total. The complete CeHR medium contained approximately 60 μM vitamin B3 (NA and NAM), and the vitamin B3-deficient medium was the complete medium without the addition of vitamin B3. The *p* value was calculated via Student's *t* test. **D** Representative images of worms cultured in complete medium or vitamin B3-deficient medium. Worms were stained with DAPI. The yellow circle highlights individual embryos, while the white arrow indicates the abnormal embryos. Scale bar = 20 μm. The percentage represents the ratio of worms with abnormal embryos. $N = 3$ independent experiments, comprising over 400 worms in total. **E** Population size of *C. elegans* cultured in complete medium, vitamin B3-deficient medium or vitamin B3-deficient medium supplemented with 60 μM NAM, NA, or NR. $N = 3$ independent experiments, each containing 3 replicates. The *p* value was calculated via one-way ANOVA followed by Tukey's multiple comparisons test. The error bars are the SEM.

manipulation but greatly limits the precise evaluation of individual nutrients in *C. elegans* because of the complex composition and metabolism of bacteria. Accordingly, a semi-chemically defined axenic medium, called *C. elegans* Habitation and Reproduction (CeHR) medium, has been developed; this medium reduces the complexities of the food input and provides controllable conditions for nutritional studies in *C. elegans*[10,11].

In the present study, we leveraged the advantages of the CeHR axenic culture system to examine the involvement of NAD⁺ biosynthetic pathways in *C. elegans* physiology and investigated the efficacy and independent nature of the two synthesis pathways. We found that the deprivation of NAD⁺ salvage precursors from the medium resulted in severe developmental delay and fecundity loss in *C. elegans*, even in the presence of tryptophan. Supplementation with intermediates from the kynurenine pathway failed to reverse fecundity defects in NAD⁺-deficient *C. elegans*. Furthermore, we unexpectedly discovered that commercially available QA products were able to rescue these defects due to contamination by substantial amounts of NA, whereas purified QA did not have the same effect. This raises concerns for future studies on NAD⁺ that employ such QA products. Additionally, our results obtained from the *umps-1* mutant provided further evidence that UMPS-1 does not play a significant role in NAD⁺ biosynthesis, as NAD⁺ levels remained unchanged and irreversible defects persisted with or without NAD⁺ salvage supplementation in these mutant animals. Taken together, these findings demonstrate that, if it exists, the proposed de novo synthesis pathway is ineffective for supplying NAD⁺ in worms.

Results

The NAD⁺ supply from the salvage pathway is indispensable for the fecundity of *C. elegans*

To test the bioavailability of NAD⁺ biosynthetic pathways in *C. elegans*, we removed two NAD⁺ precursors for salvage synthesis (NAM and NA, as indicated in Fig. 1A, referred to as vitamin B3) from the complete CeHR medium and evaluated the development and reproduction of the cultured worms. We observed that the removal of NAD⁺ salvage precursors from the medium resulted in severe developmental delay and a significant decrease in fecundity in *C. elegans* (Fig. 1B, C). Compared with those of worms cultured in the complete medium, the brood sizes of the worms cultured in the vitamin B3-deficient medium decreased markedly (Fig. 1C). Using DAPI staining, we demonstrated that embryonic deformities, indicated by the clumping of nuclei, caused a decrease in the fecundity of vitamin B3-deficient worms (Fig. 1D). Consequently, the population size of the worms cultured in the vitamin B3-deficient medium was also significantly lower

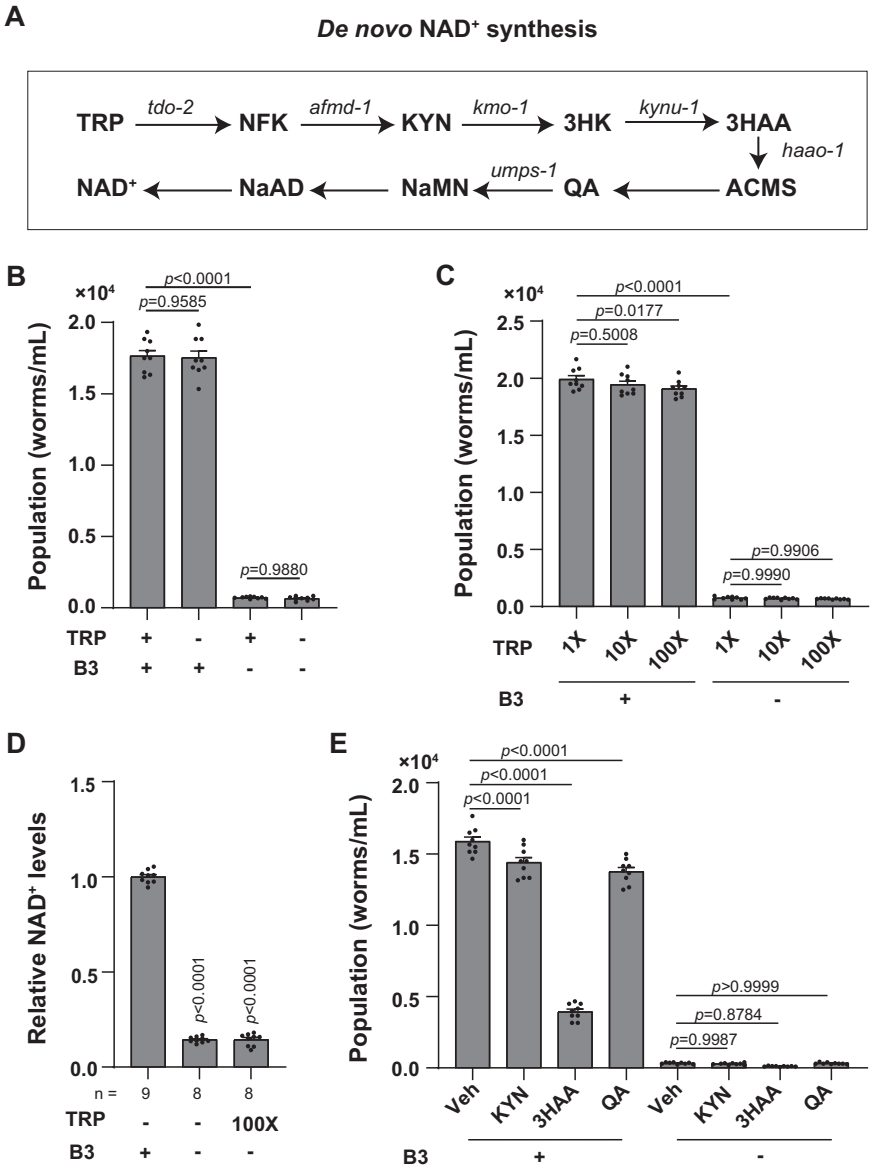

Fig. 2 | Metabolites for de novo NAD$^+$ synthesis do not contribute to the NAD$^+$ supply. A Schematic diagram of de novo NAD$^+$ synthesis in *C. elegans*. **B** Population size of worms in complete and vitamin B3-deficient media with or without 50 μM tryptophan. $N = 3$ independent experiments, each containing 3 replicates. **C** Population size of worms in complete and vitamin B3-deficient media supplemented with tryptophan (1× Trp; 10× Trp; 100× Trp; 50 μM; 500 μM; and 5 mM tryptophan). $N = 3$ independent experiments, each containing 3 replicates. **D** NAD$^+$ levels of worms in complete medium, vitamin B3-deficient medium and vitamin B3-deficient medium supplemented with tryptophan. $N = 3$ independent experiments, each containing 2-3 replicates. **E** Population size of worms in full and vitamin B3-deficient media supplemented with 10 mM intermediate for de novo NAD$^+$ synthesis. KYN, 3HAA, QA are kynurenine, 3-hydroxyanthranilic acid and quinolinic acid, respectively. $N = 3$ independent experiments, each containing 3 replicates. The error bars represent the SEM. *p* values were calculated via two-way ANOVA.

than that of the worms cultured in the full medium (Fig. 1E). However, this decrease in population size could be reversed in a dose-dependent manner by supplementation with all three forms of salvage NAD$^+$ precursors, including NR, which was not included in the original CeHR medium recipe (Fig. 1E). Similar to worms cultured in vitamin B3-deficient medium, the *pnc-1* mutant, which is impaired in salvage synthesis from NAM to NA (Fig. 1A), showed a severe decrease in fecundity in medium supplemented with NAM as the sole salvage NAD$^+$ precursor (Supplementary Fig. 1). Together, these results reveal that the availability of NAD$^+$ through salvage synthesis plays a vital role in the development and reproduction of *C. elegans*.

De novo NAD$^+$ synthesis does not contribute to the supply of NAD$^+$ in *C. elegans*

Tryptophan serves as the primary precursor of the de novo synthesis pathway for NAD$^+$ (Fig. 2A). The vitamin B3-deficient medium, as well as the complete medium, contained 50 μM tryptophan, a dose that has been shown to significantly increase NAD$^+$ levels in worms fed with bacteria[4]. However, the removal of tryptophan from the medium did not affect the population size in either complete or vitamin B3-deficient medium (Fig. 2B). To explore whether the ineffectiveness of tryptophan was due to insufficient amounts, we increased the concentration of tryptophan in the vitamin B3-deficient medium and tested worm reproduction. Unexpectedly, even with supplementation with tryptophan at concentrations 10-fold (500 μM) or 100-fold (5 mM) greater than that in the standard medium, the fecundity or NAD$^+$ levels of worms cultured in the vitamin B3-deficient medium could not be restored (Fig. 2C, D). Notably, the expressions of genes related to the kynurenine pathway and *umps-1* were slightly or significantly increased in these worms compared with that in worms fed with bacteria (Supplementary Fig. 2A). It has been reported that increased expression of kynurenine pathway genes can lead to elevated production of QA[12]. This may theoretically elevate NAD$^+$ levels, provided the de novo NAD$^+$ synthesis mechanisms are functional. Consequently, we investigated whether other intermediates from the kynurenine pathway could rescue the low fertility of vitamin B3-deficient worms. These intermediates included kynurenine (KYN), 3-hydroxyanthranilic acid (3-HAA), and purified QA, the successful supplementation of which have been verified by LC-MS/MS (Fig. S2B–G). Similar to the outcomes of tryptophan administration, supplementation of these intermediates did not restore the fecundity of worms cultured in vitamin B3-deficient

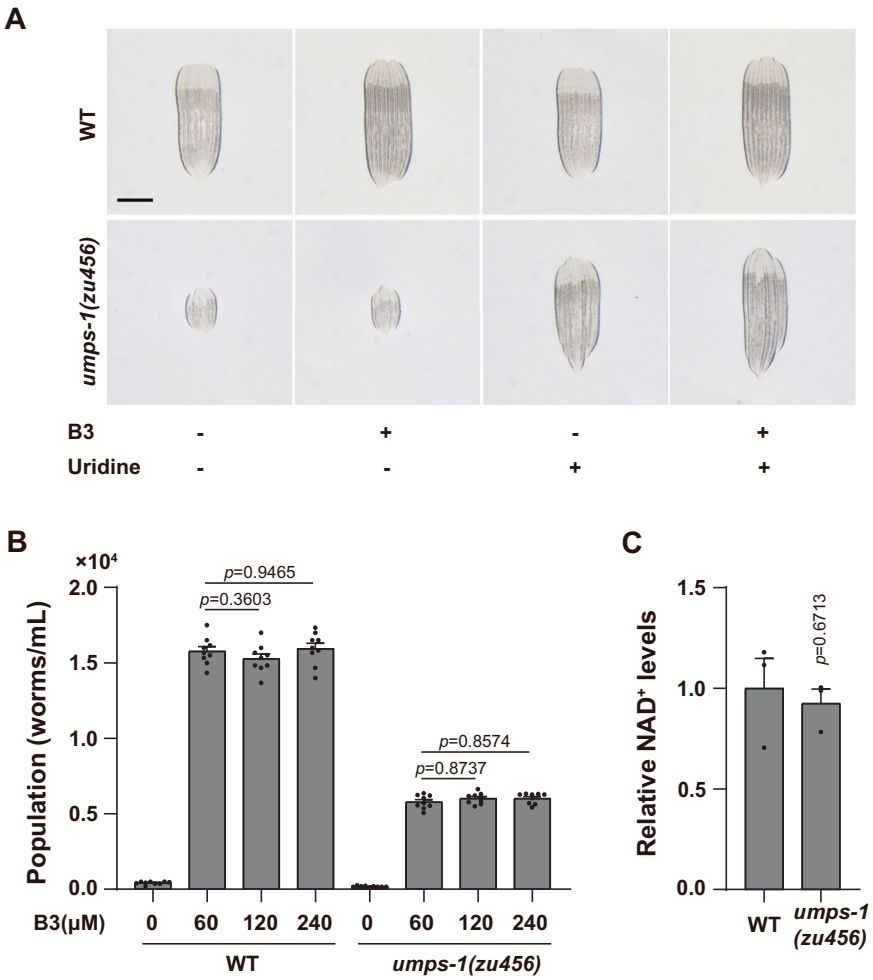

Fig. 3 | Depletion of UMPS-1 does not induce changes in reproduction regulated by NAD$^+$. **A** Representative images of N2 and *umps-1* mutant worms cultured in vitamin B3-deficient medium and complete medium with or without additional 10 mM uridine. Scale bar = 200 μm. **B** Population size of *umps-1* worms in complete and vitamin B3-deficient media with an additional vitamin B3 mixture. $N = 3$ independent experiments, each containing 3 replicates. The p value was calculated via two-way ANOVA. **C** NAD$^+$ levels of N2 and *umps-1* mutant worms in complete medium. $N = 3$ independent experiments. The p value was calculated via Student's t test. The error bars are the SEM.

medium; instead, it resulted in a reduced fecundity in the complete medium (Figs. 2E, S3A). Additionally, these supplementations did not recover either the development or NAD$^+$ levels of vitamin B3-deficient worms (Fig. S3B, S3C). To investigate whether these results are specific to the axenic culture system, we supplied the purified QA to worms fed with bacterial food and demonstrated the unchanged NAD$^+$ levels in this setting (Supplementary Fig. 3D).

Notably, we initially observed a significant rescue of NAD$^+$ deficiency-related defects in worms when commercially purchased QA products were used at very high concentrations (Supplementary Fig. 4A). Considering the structural similarity between QA and NA (Supplementary Fig. 4B), we speculated that potential NA contamination in these QA products might be responsible for their rescue effects. Consistent with our hypothesis, we found that NA was commonly present in all the tested QA products, at proportions ranging from 20-100 μM per 64 mM QA (Supplementary Fig. 4C). Interestingly, when we removed the NA impurities from QA, the purified QA failed to rescue the NAD$^+$ deficiency (Figs. 2E, S4D, and S4E), which raises important concerns for future studies involving the use of these commercially available QA products. Overall, our findings highlight the ineffectiveness of de novo NAD$^+$ synthesis in supplying NAD$^+$ in *C. elegans*.

Depletion of UMPS-1 does not induce changes in reproduction regulated by NAD$^+$ and NAD$^+$ levels

Previous studies have shown that de novo NAD$^+$ synthesis can occur through the action of UMPS-1 in *C. elegans*[9]. UMPS-1 is an essential enzyme for pyrimidine synthesis. Consistent with the classic functions of UMPS-1, the *umps-1* mutant presented severe developmental defects in both full medium and vitamin B3-deficient medium, whereas the addition of uridine, but not vitamin B3, effectively rescued the developmental defect phenotype (Fig. 3A). These findings confirm the functional role of UMPS-1 in wild-type worms and the complete loss of function in *umps-1* mutants. Depletion of *umps-1* was reported to decrease the fecundity of worms[9]. In the axenic culture system, we also observed a significant decrease in the fecundity of the *umps-1* mutant, even with uridine supplementation (Fig. 3B). This decrease could not be alleviated by supplementation with vitamin B3 (Fig. 3B). Additionally, the NAD$^+$ levels in the *umps-1* mutant did not decrease compared with those in the wild type (Fig. 3C). These results consistently suggest that the fecundity reduction in *umps-1* mutants is not due to NAD$^+$ deficiency. Instead, this reduction is likely attributable to the accumulation of orotic acid, an upstream metabolite of UMPS-1, which has been reported to cause embryonic lethality[13]. These findings demonstrate that UMPS-1 does not contribute to the NAD$^+$ supply in worms and further prove the ineffectiveness of de novo NAD$^+$ synthesis in *C. elegans*.

Discussion

The levels of NAD$^+$ decline gradually with age in both mammals and invertebrates, and this decline has been causally connected to various aging-related diseases, including metabolic disease, cancer and cognitive decline[2,14]. Accordingly, interest in the promotion of healthy aging through the modulation of NAD$^+$ levels has increased in recent decades[2], which strongly necessitates a comprehensive understanding of NAD$^+$ biogenesis pathways as well as their efficacy.

C. elegans has been widely used in studies on metabolism and aging because of its short lifespan and well-conserved metabolic

pathways. Numerous studies have revealed that NAD^+ contributes to various aspects of worm physiology, including mitochondrial function, metabolism, and neuronal activity[4,14–19]. The distinct roles of NAD^+ biosynthetic pathways have also been explored in *C. elegans*[4,9]. However, nearly all studies on NAD^+ have been conducted in *C. elegans* fed with bacteria, which are difficult to make metabolically inactive even after heat- or UV-killing treatment[20]. For example, *pnc-1* mutant worms present severe defects in axenic culture (Supplementary Fig. 1) but only mild defects when fed with bacteria in regular culture[18]. This mild defect on NGM plates could be attributed to interference from bacterial metabolism, as bacteria contain various precursors for salvage NAD^+ synthesis. Additionally, bacteria may produce secondary metabolites, leading to different outcomes[21]. For instance, bacteria have been shown to enhance host NAD^+ metabolism by engaging the deamidated biosynthesis pathway. The bacterial PncA enzyme converts NAM into NA, which supports host NAD^+ synthesis[22].

Here, we applied chemically defined CeHR medium to evaluate the role and efficacy of NAD^+ biosynthetic pathways in supporting the physiology of *C. elegans*, which allowed the creation of conditions undisturbed by bacteria and the convenient creation of a deficient medium[10,11,23]. It has been reported that this CeHR enables a growth rate in worms closer to that on NGM plates compared to other chemically defined media, such as *C. elegans* maintenance medium (CeMM) and axenic medium (AXM)[24]. A recent study also reported that CeHR downregulates genes involved in reproduction and oogenesis, which may account for the reduced fecundity of worms cultured in CeHR[25]. Under this culture condition, we demonstrated that vitamin B3 deficiency results in significant developmental and reproductive defects in *C. elegans*, even when tryptophan or intermediates from the kynurenine pathway are present in substantial quantities. Consistent with our observations, genetic deficiency of *nmat-2*, which encodes a rate-limiting enzyme for NAD^+ synthesis, was also associated with developmental delays and sterile phenotypes[16]. These results collectively indicate that the NAD^+ supply from the salvage pathway is indispensable for worm physiology, whereas de novo synthesis is ineffective in *C. elegans*.

Tryptophan-derived NAD^+ is sufficient to meet NAD^+ demands under vitamin B3 deficiency in mammals[7]. In contrast, in *C. elegans*, in which the classic QPRTase is absent, precursors for de novo NAD^+ synthesis cannot compensate for NAD^+ deficiency. The loss of QPRTase in *C. elegans* during evolution could be partly explained by its primary food source being bacteria, which have an abundance of salvage NAD^+ precursors and are sufficient for maintaining worm physiology. Although UMPS-1 has decarboxylase and phosphoribosyltransferase activity, it did not exhibit efficient QPRTase activity in our study. Moreover, the ability of UMPS-1 to perform QPRTase function has not been demonstrated in other species.

Impurities in compounds or drugs pose significant problems in biological research. The presence of impurities in a drug could result in potential safety issues, such as toxicity and adverse immunostimulation[26,27], as well as impact the drug's activity[28]. Furthermore, these impurities may lead to altered or even erroneous experimental results. For example, a recent study reported that the presence of copper impurities in iron raw material altered the performance of cell culture[29]. Occasionally, in this study, we also discovered that a substantial amount of NA impurities in QA significantly influenced the phenotypes of the worms. These findings emphasize the importance of carefully considering the potential impact of impurities on experimental outcomes, especially when compounds are used at high concentrations.

Our study revealed that de novo NAD^+ synthesis for NAD^+ supply is ineffective in *C. elegans* and that UMPS-1 does not significantly contribute to NAD^+ homeostasis. Therefore, future research targeting the de novo NAD^+ synthesis pathway for the modulation of NAD^+ levels in *C. elegans* should be conducted with more caution.

Materials and Methods

Key resource table

Reagent or resource type	Designation	Source or reference	Identifiers	Additional information
Strain, strain background (*C. elegans*)	N2	CGC	N2	Wildtype
Strain, strain background (*C. elegans*)	WLU155	This paper	WU155[pnc-1(aly36)]	*pnc-1* mutant
Strain, strain background (*C. elegans*)	GH636	CGC	GH636[umps-1(zu456)]	*umps-1* mutant
Strain, strain background (*Escherichia coli*)	OP50	CGC	N/A	
Chemical compound, drug	NAM	Sigma	N3376	
Chemical compound, drug	NA	Sigma	N0761	
Chemical compound, drug	NR	MACKLIN	N885871	
Chemical compound, drug	Tryptophan	Sigma	T8941	
Chemical compound, drug	Kynurenine	MACKLIN	L864410	
Chemical compound, drug	3-hydroxyanthranilic acid	MACKLIN	A842242	
Chemical compound, drug	Uridine	Sigma	U3003	
Chemical compound, drug	Quinolinic acid	Sigma	P63204	
Chemical compound, drug	Quinolinic acid	Macklin	P815409	
Chemical compound, drug	Quinolinic acid	Solarbio	Q0100	
chemical compound, drug	Quinolinic acid	MCE	HY-100807	
Chemical compound, drug	Quinolinic acid	ABMole	M7178	
Chemical compound, drug	Purified QA	This paper	N/A	From Yuhao Chemical company (Hangzhou)
Commercial assay or kit	5 × Hiscript ® III QRT SuperMix	Vazyme	R323-01	
Commercial assay or kit	ChamQ Universal SYBR qPCR Master Mix	Vazyme	Q711-02	
Software, algorithm	GraphPad Prism 8	GraphPad	Prism 8.3.0	
Others	DAPI	Beyotime	C1002	
Sequence-based reagent	*act-1* Forward	This paper	qPCR primers	TGCTGA TCGTATG CAGAAGG
Sequence-based reagent	*act-1* Reverse	This paper	qPCR primers	TAGATC CTCCGAT CCAGACG
Sequence-based reagent	*tdo-2* Forward	This paper	qPCR primers	ACCAGC TTCGGGA TTCCAGA
Sequence-based reagent	*tdo-2* Reverse	This paper	qPCR primers	TGATGG GTCCGCT GCTTGTT
Sequence-based reagent	*afmd-1* Forward	This paper	qPCR primers	GCAATG AACGCTG TTGCACG
Sequence-based reagent	*afmd-1* Reverse	This paper	qPCR primers	GCCCAC TGTTTGG AAACTCC
Sequence-based reagent	*kmo-1* Forward	This paper	qPCR primers	CCCACC ACAAGAG CATGCTG
Sequence-based reagent	*kmo-1* Reverse	This paper	qPCR primers	AGTGAA ATGACCT CGTGGCC

Sequence-based reagent	*kynu-1* Forward	This paper	qPCR primers	CCAGAT GCTGATC CAAGCCT
Sequence-based reagent	*kynu-1* Reverse	This paper	qPCR primers	AGGAAG TCAAGAG CACGTGG
Sequence-based reagent	*haao-1* Forward	This paper	qPCR primers	GTTCAT GTTGCCA GCAAGAGT
Sequence-based reagent	*haao-1* Reverse	This paper	qPCR primers	CTCACT GGTCCGT TTTTCAC
Sequence-based reagent	*acsd-1* Forward	This paper	qPCR primers	CTGCAA CCTCAAA ATCGCGG
Sequence-based reagent	*acsd-1* Reverse	This paper	qPCR primers	CTGTAT CGAAGC AGTTGGGC
Sequence-based reagent	*umps-1* Forward	This paper	qPCR primers	CCGGGG CTTTTGA TGCTTAT
Sequence-based reagent	*umps-1* Reverse	This paper	qPCR primers	GCTTCC ACCTGTC GTCACGA

C. elegans strain and maintenance

The *C. elegans* strains used in this study were maintained on nematode growth medium (NGM) plates at 20 °C with *E. coli* OP50 as a food source before experiments were conducted in the axenic culture system. The following strains were used in this research: N2, GH636 [*umps-1*(*zu456*)], and WLU155 [*pnc-1*(*aly36*)]. GH636 contain a AT to TA nucleotide change in the start codon of *umps-1*(ATG to TAG), which theoretically results in a null allele of *umps-1*.

Axenic culture

The CeHR medium was prepared following a previously described protocol[10] and contained 50 μM tryptophan and ~60 μM NAM and NA (Supplementary Data 1). Deficient medium was prepared without the indicated corresponding compounds. Synchronized L1 worms were cultured in CeHR medium with continuous shaking at 70 rpm on a shaker at 20 °C. Synchronization was conducted according to the hypochlorite method to remove bacteria.

Supplementation of NAD^+ synthesis precursors

NAM (Sigma, N3376), NA (Sigma, N0761) and NR (MACKLIN, N885871) were prepared as 6 mM stock solutions and then added to CeHR medium at a final concentration of 60 μM. A 55 mM stock solution of tryptophan (Sigma, T8941) was added to the CeHR medium at working concentrations of 5 mM and 500 μM. Stock solutions of KYN (MACKLIN, L864410) and 3-HAA (MACKLIN, A842242) were prepared at 100 mM. Stock solutions of QA (Sigma, P63204; MACKLIN, P815409; Solarbio, Q0100; MCE, HY-100807; ABMole, M7178) were prepared at 500 mM. The purified QA was obtained from Yuhao Chemical Company (Hangzhou). The pH of KYN, 3-HAA, and QA was neutralized with NaOH to 7.0.

Supplementation of QA on NGM plates

N2 worms were cultured on NGM plates until the L4 stage, then transferred to NGM plates containing UV-killed bacteria supplemented with 10 mM QA or vehicle. After reaching the young adult stage, ~10,000 worms were collected for further LC-MS detection. NGM plates with UV-killed bacteria were prepared by subjecting the bacteria to 10 min of UV treatment.

DAPI staining

Worms cultured for 120 h were fixed with precooled 100% methanol. The fixed worms were stained with 1 μg/mL DAPI for 20 min at 4 °C, washed with M9 buffer three times and mounted on a 2% agarose pad. The maximum intensity projection Z-stack images of the stained worms were acquired with a Zeiss LSM800.

Developmental assay

L1 worms were cultured in 500 μL of CeHR medium in a 24-well plate for 84 h. Before the evaluation of developmental stages, the worms were washed with M9 buffer containing 0.05% Triton X-100 three times, paralyzed with 25 mM NaN_3 and mounted on a 2% agarose pad. Developmental stages were determined on the basis of vulvar morphology via microscopy.

Population size measurement

The population size was evaluated by the total number of worms produced from 100 L1s cultured in 1 mL of CeHR for 10 days. Worm cultures were diluted with M9 buffer containing 0.05% Triton X-100 before counting. Each sample was counted three times, and the results were averaged. All the experiments were conducted with three biological replicates.

Brood size

Synchronized L1 worms were cultured in CeHR medium for 60 hours before being individually seeded into a 96-well plate with 100 μL of CeHR medium per well. Eight worms were tested for each condition within 8 days. The tested worms were transferred to new wells every day, and the number of eggs laid in each well was counted every day until no more eggs were laid. All operations were carried out in a laminar flow hood to avoid possible contamination. All the experiments were conducted three times.

RNA extraction and quantitative RT-PCR

Total RNA from young adult worms was extracted with an Easy RNA kit (Easy-do DR0401050). The removal of gDNA and reverse transcription of worm RNA were performed with HiScript III RT SuperMix for qPCR (+gDNA wiper) (Vazyme, R323). Quantitative PCR was conducted with ChamQ Universal SYBR qPCR Master Mix (Vazyme, Q711) on a quantitative PCR system (Jena Qtower 3G). The expression levels of genes of interest were normalized to those of *act-1*.

LC-MS/MS measurement of NAD^+ levels

The NAD^+ levels of the worms were determined via LC-MS/MS. Worms cultured on NGM or in axenic culture with the corresponding treatments until young adults were obtained. Approximately 10,000 young adult worms were collected and flash frozen in liquid nitrogen. The samples were resuspended in a solution containing acetonitrile:methanol:water (2:2:1) and 0.1 M formic acid and then homogenized with a sonication device (Sonics VCX150 Ultrasonicator) on ice. The homogenized samples were neutralized with 43.5 μL of 15% NH_4HCO_3 per 500 μL of sample. After 30 min of extraction at -80 °C, the samples were then centrifuged at 12,000 rpm for 30 min at 4 °C. The supernatant was collected and subjected to LC-MS/MS detection. LC-MS/MS analysis was conducted via an AB SCIEX QTRAP 6500+ mass spectrometer connected to an Exion LC system. Chromatographic separation was achieved on a hypercarb column (50 mm × 4.6 mm, 3 μm) at 40 °C. Mobile phase A was ultrapure water containing 0.3% ammonia and 15 mM ammonium acetate, and mobile phase B was acetonitrile/water (9:1) containing 0.3% ammonia and 15 mM ammonium acetate. The flow rate was 0.4 mL/min, and the gradient of mobile phase B was as follows: 5% in 0.5 min, 5-90% in 3.5 min, held at 90% for 1 min, 90-5% in 0.1 min, and held at 5% for 1.9 min. The sample volume injected was 5 μL. The mass spectrometer was operated in positive ion mode with the following settings: curtain gas, 40 psi; ion spray voltage, 5.5 kV; source temperature, 550 °C; ion source gas, 160 psi; and ion source gas, 255 psi. The compounds were measured via multiple reaction monitoring (MRM) with an optimized cone voltage and collision energy. The protein pellet from worms was dissolved in 1 mL of 1% SDS for the BCA assay and subsequently used for normalization.

LC-MS/MS measurement of NA

QA solutions (100 mM) of different brands and NA samples of different concentrations used for standard curve preparation were

subjected to LC-MS/MS detection. LC-MS/MS analysis was conducted via an AB SCIEX QTRAP 6500+ mass spectrometer connected to an Exion LC system. All the data were collected and processed via Analyst software. Chromatographic separation was achieved on a Waters ACQUITY UPLC BEH amide (50 mm × 2.1 mm, 1.7 μm) column. The mobile phase consisted of 10 mM ammonium acetate and 0.05% (v/v) ammonia in water as solvent A and 10 mM ammonium acetate and 0.05% (v/v) ammonia in 90% (v/v) acetonitrile as solvent B at a flow rate of 0.4 mL/min. The gradient of mobile phase B was as follows: 95% for 1 min, 95-55% for 1.5 min, 55% for 2.5 min, 55-95% for 0.1 min, and 95% for 2.9 min. The flow rate of the mobile phase was 0.4 mL/min, the column temperature was 40 °C, and the injection volume was 1 μL. The mass spectrometer was operated in positive ion mode with the following settings: curtain gas, 35 psi; ion spray voltage, 5.5 kV; ion source temperature, 500 °C; ion source gas 1, 55 psi; and ion source gas 2, 50 psi. The quantitative analysis of NA was performed in multiple reaction monitoring (MRM) mode. The MRM transition used was m/z 124.1 → 80.

LC-MS/MS measurement of metabolites in the kynurenine pathway

The metabolite levels in the worms were determined using LC-MS/MS. Worms were cultured in axenic conditions with the corresponding treatments until they reached the young adult stage. Approximately 10,000 young adult worms were collected and flash-frozen in liquid nitrogen. The samples were resuspended in 250 μL of methanol:water (4:1) and homogenized on ice using a sonication device (Sonics VCX150 Ultrasonicator). The samples were then centrifuged at 12,000 rpm for 30 min at 4 °C. The supernatant was collected and subjected to LC-MS/MS analysis. LC-MS/MS analysis was conducted using AB SCIEX QTRAP 6500+ mass spectrometer connected with Exion LC system. All data were collected and processed using the Analyst Software. Chromatographic separation was achieved on a Waters ACQUITY UPLC BEH T3 (100 mm × 2.1 mm, 1.8 μm) column. The mobile phase consisted of 0.1% (v/v) formic acid in water as solvent A and 0.1% (v/v) formic acid in acetonitrile as solvent B at a flow rate of 0.4 mL/min. The gradient of mobile phase B was 5% in 1 min, 5-95% in 1.5 min, held at 95% for 2.5 min, then 95-5% in 0.1 min, held at 5% for 2.9 min. The flow rate of mobile phase was kept at 0.4 mL/min, column temperature was set at 40 °C and the injection volume was 1 μL. Mass spectrometer was operated in positive ion mode (ion spray voltage 5.5 kV) for quantification of TRP, KYN, KYAC, and negative ion mode (ion spray Voltage -4.5 kV) for quantification of QA and 3HAA, with the following settings: curtain gas, 35 psi, ion source temperature, 500 °C, ion source gas 1, 55 psi, ion source gas 2, 50 psi. The quantitative analysis of metabolites was measured by MRM mode. The protein pellet from worms was dissolved in 1 mL of 1% SDS for the BCA assay and subsequently used for normalization.

Imaging of *umps-1* mutant worms

Synchronized L1 worms of N2 or *umps-1* mutants were cultured in CeHR medium for 84 h. Worms were cultured with or without 10 mM uridine (Sigma, U3003). Worms were paralyzed with 25 mM NaN_3 and placed on 2% agarose pads. Images of the worms were acquired with a Nikon SMZ18.

Statistics and reproducibility

The data were analyzed with GraphPad Prism 8 (GraphPad Software, Inc.). p values of differences between two groups were calculated, and an unpaired Student's t test was used. p values among multiple groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Two-way ANOVA was used to analyze differences between multiple groups with two variations, followed by Sidak's or Tukey's multiple comparisons test. The error bars are the SEM. All results were obtained from at least three biological experiments. The sample size was provided in the figures or figure legends.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All the data are included within this manuscript. The source data for all presented figures were provided in Supplementary Data 2.

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Author contributions

S.Z., R.Z., and L.W. designed the experiments. S.Z. conducted most of the experiments and data analysis. R.Z., L.Y., Z.L., Y.L., and S.L. carried out part of the experiments and participated in data discussions. L.W. supervised the project. S.Z., R.Z., and L.W. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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