Cell Metabolism
Previews

CellPress

The NARly side of whole-body NAD homeostasis

Charles Brenner[1,*]
[1]Beckman Research Institute of City of Hope, Duarte, CA 91010, USA
*Correspondence: cbrenner@coh.org
https://doi.org/10.1016/j.cmet.2025.06.003

Nicotinic acid riboside (NAR), one of two nucleoside precursors of nicotinamide adenine dinucleotide (NAD) coenzymes, is revealed to function in systemic NAD homeostasis. By knocking out *Nmnat1* in liver, investigators discovered a liver-to-kidney NAR transit pathway and learned that kidney can be a donor in addition to a receiver of NAD precursors.

Four nicotinamide adenine dinucleotide (NAD) coenzymes differing in redox state and by adenosine 2′-phosphorylation (i.e., NAD^+, NADH, $NADP^+$, and NADPH) function as the central catalysts of life by conveying high-energy electrons in fuel oxidation, ATP generation, biosynthesis reactions, and both the generation and detoxification of reactive oxygen species.[1] In mammals, NAD coenzymes are maintained with a combination of *de novo* and salvage biosynthesis reactions that function both cell autonomously and in relationship to other cells. In a first dimension, homeostasis in the NAD system involves the liver's responding to high availability of one precursor by boosting the hepatic NAD metabolome with distribution of other NAD precursors[2,3] and metabolites.[3] In a second dimension, homeostasis in the NAD system involves insults to this system that result in induction of gene expression programs that attempt to restore the NAD metabolome.[4] In a third dimension, microbial systems running in the background can convert one metabolite to another, thereby facilitating host-NAD system homeostasis.[5] All of the known and unknown dimensions of NAD regulation run simultaneously, awaiting clever research groups to perform new perturbations and analyses that reveal the mechanisms behind the curtain.[6]

Everywhere in biology, there are *de novo* and salvage biosynthetic programs for complex biochemicals. Logically, there have to be *de novo* programs to make the unique parts that are salvaged. In mammalian NAD biosynthesis, hepatocytes conduct *de novo* synthesis from tryptophan in an 8-enzymatic step pathway that can run cell autonomously. As shown in Figure 1, the sixth *de novo* step forms nicotinic acid mononucleotide (NAMN) from quinolinic acid. Conventional wisdom saw this mononucleotide as fully committed to NAD synthesis. Though it was known that some of the liver's NAD biosynthetic program supports other tissues through nicotinamide (NAM) circulation,[2] it was not known that liver can dephosphorylate NAMN to nicotinic acid riboside (NAR) for distribution to other tissues or that the kidney is not only a receiver but a donor of precursors.[6]

Due to (1) NAM circulation, (2) intracellular production of NAM by NAD-consuming enzymes,[1] and (3) dietary NAM as a major NAD coenzyme breakdown product, NAM is the central and most common salvageable precursor of NAD. It appears that every mammalian cell expresses nicotinamide phosphoribosyltransferase (*Nampt*) to convert NAM to nicotinamide mononucleotide (NMN) to regenerate NAD coenzymes. Because fungi and bacteria in microbiomes and in the food chain convert NAM to nicotinic acid (NA),[5,7] many mammalian cells also express nicotinic acid phosphoribosyltransferase (*Naprt*) for NA salvage. Just as *Nampt* produces NMN and requires a nicotinamide mononucleotide adenylyltransferase (Nmnat) isozyme to produce NAD, *Naprt* produces NAMN, which requires an Nmnat isozyme to produce nicotinic acid adenine dinucleotide (NAAD) to regenerate NAD coenzymes (Figure 1).

On the basis that tiazofurin and benzamide riboside are toxic analogs of nicotinamide riboside (NR) that are converted to toxic analogs of NMN and NAD, it was predicted that mammalian cells must have an enzyme for NR salvage—this led to identification of NR kinase 1 (NMRK1) and 2 (NMRK2) as the enzymes that catalyze NR conversion to NMN.[8] The crystal structure of human NMRK1 and biochemical work with both NMRK1 and NMRK2 established that these enzymes can use NR or NAR to produce NMN and NAMN,[9] respectively. Thus, NAM, NR, NA, and NAR are the salvageable precursors that feed into production of NMN and NAMN to regenerate NAD coenzymes. NAMN is at an interesting juncture in NAD metabolism because it is formed in *de novo* synthesis and in salvage synthesis from both NA and NAR (Figure 1).

Whereas the existence of NAM and NA salvage pathways is justified by enzymatic reactions that produce these bases, the NR pathway was found on the basis of toxic NAD prodrug biosynthesis[8] such that it was not obvious what would be the endogenous sources of nucleoside substrates of NMRK1 and NMRK2. As a rule, cells produce nucleotides, not nucleosides, as building blocks for nucleoside triphosphates, synthesis of nucleic acids, and synthesis of NAD coenzymes. For example, whereas adenosine signaling is well known, adenosine is not synthesized directly or intracellularly for this purpose. Rather, adenosine is generated by intracellular production and release of ATP, and followed by extracellular phosphate removal.

Thus, while identification of the NR pathway also enabled discovery of disturbances to the pathway such as massive upregulation of *Nmrk2* in heart failure,[4] it has not been clear where the nucleosides entering this pathway come from except when they are provided by exogenous NR supplementation.[3] Just like *Nampt*, *Nmrk1* appears to be ubiquitously expressed in every cell type, whereas *Nmrk2* is largely confined to muscles and is inducible by damage.[4] It seems implausible that these genes would be

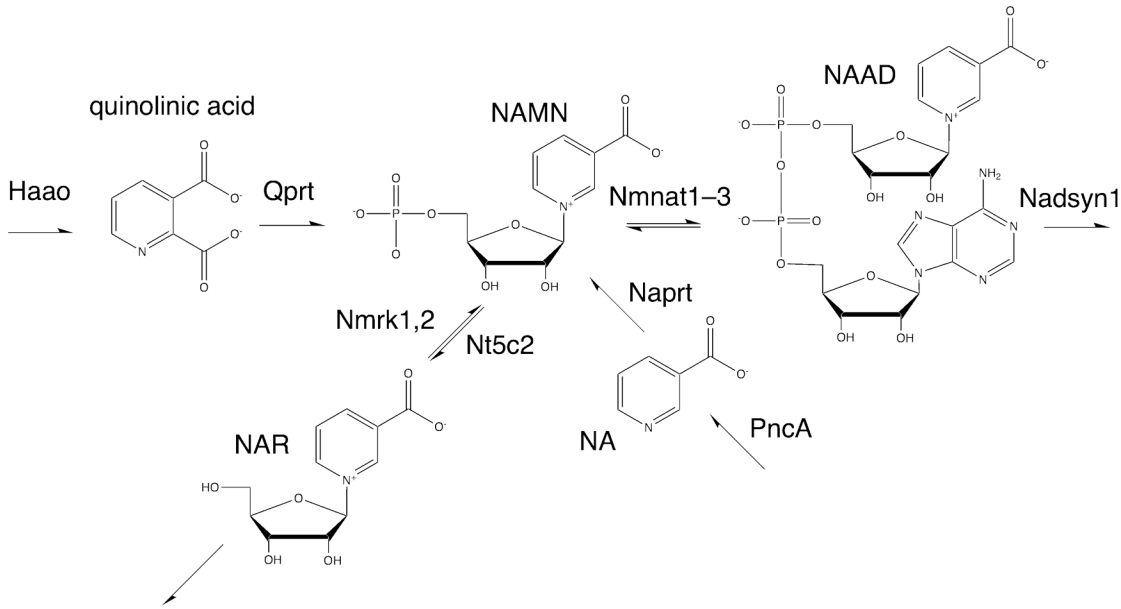

Figure 1. Generaton of NAR from NAMN in liver

The schematic shows conversion of quinolinic acid to NAMN to NAAD with Nmnat1–3 depicted as a reversible step. Haao, Qprt, Nmnat1–3, and Nadsyn1 catalyze the fifth through eighth steps in the *de novo* NAD synthesis pathway from tryptophan. NAMN is also the product of anabolic reactions from NA and NAR catalyzed by Naprt and Nmrk1,2. NA is the product of bacterial and/or fungal PncA. NAMN synthesis is shown as reversed by Nt5c2 in a process that would make NAR available to other cells when NAMN levels are high.

ubiquitously expressed or specifically regulated unless there are endogenous and possibly inducible sources of NR and/or NAR. Whither the sources?

Complicating matters, purine nucleoside phosphorylase (Pnp), which is normally intracellular, is partially extracellularized in blood draws such that Pnp artifactually degrades NR to NAM plus ribose-1-phosphate.[10] At neutral pH, NR is a cation that is briskly degraded by Pnp and uridine hydrolase, whereas NAR is a zwitterion that is substantially more stable to enzymatic and nonenzymatic degradation.[10]

Song and coworkers[6] did not specifically set out to determine the endogenous sources of NR and/or NAR. Rather, they wanted to see what would happen when they deleted the major Nmnat isozyme, *Nmnat1*, in liver. With depression of hepatic NAD^+, they saw elevation of liver NMN and NAMN and discovered a huge increase in hepatic and circulating NAR.[6] By viral knockdown, the step of conversion of accumulated NAMN to NAR was found to be catalyzed by a 5′-nucleotidase encoded by the *Nt5c2* gene, and the major tissue receiving the NAR was found to be the kidney. Further, they showed that levels of NAR decline in aging while provision of supplementary NAR supports a newfound ability of the mouse kidney to circulate NAM.[6] Of potential translational significance, supplementary NAR also supported mouse kidney function in aging.[6]

While it has been claimed that production of deamidated NAD metabolites depends entirely on the gut microbiome,[5] it has also been proposed that boosting NAD in liver directly produces flux from NAD to NAAD and NAMN.[3] This new work supports that interpretation and additionally suggests that NR may be circulated by liver, though it is very difficult to detect NR after blood draws and sample handling. The endogenous sources of NR or NAR in conditions such as heart failure in which one or both *Nmrk* genes are induced remain a mystery, though new insights and tools for systemic NAD metabolism provide some pathways for the search.

ACKNOWLEDGMENTS

Work was supported by the Alfred E. Mann Family Foundation.

DECLARATION OF INTERESTS

C.B. is a shareholder and advisor of Niagen Bioscience, Alphina Therapeutics, Juvenis, and NADMED.

REFERENCES

1. Belenky, P., Bogan, K.L., and Brenner, C. (2007). NAD^+ metabolism in health and disease. Trends Biochem. Sci. *32*, 12–19. https://doi.org/10.1016/j.tibs.2006.11.006.

2. Bender, D.A., Magboul, B.I., and Wynick, D. (1982). Probable mechanisms of regulation of the utilization of dietary tryptophan, nicotinamide and nicotinic acid as precursors of nicotinamide nucleotides in the rat. Br. J. Nutr. *48*, 119–127. https://doi.org/10.1079/bjn19820094.

3. Trammell, S.A.J., Schmidt, M.S., Weidemann, B.J., Redpath, P., Jaksch, F., Dellinger, R.W., Li, Z., Abel, E.D., Migaud, M.E., and Brenner, C. (2016). Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. Nat. Commun. *7*, 12948. https://doi.org/10.1038/ncomms12948.

4. Diguet, N., Trammell, S.A.J., Tannous, C., Deloux, R., Piquereau, J., Mougenot, N., Gouge, A., Gressette, M., Manoury, B., Blanc, J., et al. (2018). Nicotinamide riboside preserves cardiac function in a mouse model of dilated cardiomyopathy. Circulation *137*, 2256–2273. https://doi.org/10.1161/circulationaha.116.026099.

5. Shats, I., Williams, J.G., Liu, J., Makarov, M.V., Wu, X., Lih, F.B., Deterding, L.J., Lim, C., Xu, X., Randall, T.A., et al. (2020). Bacteria boost mammalian host NAD metabolism by engaging the deamidated biosynthesis pathway. Cell Metab. *31*, 564–579.e7. https://doi.org/10.1016/j.cmet.2020.02.001.

6. Song, W.-S., Shen, X., Du, K., Ramirez, C.B., Park, S.H., Cao, Y., Le, J., Bae, H., Kim, J.,

Chun, Y., et al. (2025). Nicotinic acid riboside maintains NAD^+ homeostasis and ameliorates aging-associated NAD^+ decline. Cell Metab. *37*, 1499–1514.e4. https://doi.org/10.1016/j.cmet.2025.04.007.

7. Gazzaniga, F., Stebbins, R., Chang, S.Z., McPeek, M.A., and Brenner, C. (2009). Microbial NAD metabolism: lessons from comparative genomics. Microbiol. Mol. Biol. Rev. *73*, 529–541. https://doi.org/10.1128/mmbr.00042-08.

8. Bieganowski, P., and Brenner, C. (2004). Discoveries of nicotinamide riboside as a nutrient and conserved *NRK* genes establish a Preiss-Handler independent route to NAD^+ in fungi and humans. Cell *117*, 495–502. https://doi.org/10.1016/S0092-8674(04)00416-7.

9. Tempel, W., Rabeh, W.M., Bogan, K.L., Belenky, P., Wojcik, M., Seidle, H.F., Nedyalkova, L., Yang, T., Sauve, A.A., Park, H.-W., and Brenner, C. (2007). Nicotinamide riboside kinase structures reveal new pathways to NAD^+. PLoS Biol. *5*, e263. https://doi.org/10.1371/journal.pbio.0050263.

10. Belenky, P., Christensen, K.C., Gazzaniga, F., Pletnev, A.A., and Brenner, C. (2009). Nicotinamide riboside and nicotinic acid riboside salvage in fungi and mammals: quantitative basis for Urh1 and purine nucleoside phosphorylase function in NAD^+ metabolism. J. Biol. Chem. *284*, 158–164. https://doi.org/10.1074/jbc.M807976200.