# Two new perspectives on NAD metabolism

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**Abstract:** $NAD^+$ has gained increased attention during recent years due to its involvement in cellular signalling and regulation and changes in NAD metabolism are associated with ageing, diabetes and neurodegenerative diseases. Here we review the key findings of our two recent studies on the theoretical investigation of NAD metabolism. In the first [dFGZS11], we used elementary flux mode analysis and revealed unexpected fluxes including futile cycles and $NAD^+$ signalling without net consumption of $NAD^+$. We furthermore identified essential enzymes such as $NAD^+$-kinase (NADK), converting $NAD^+$ into $NADP^+$, and the mononucleotide adenylyl transferase (NMNAT). The second study [GZP+12], investigated the phylogeny of this pathway and revealed that the two NAD salvage pathways exist simultaneously in some species and that the first enzyme of this pathway in higher eukaryotes (Namphosphoribosyltransferase (NamPT)) seems to have been lost several times during evolution. Both analyses successfully combine bioinformatic approaches with biochemical expertise.

## 1 Introduction

$NAD^+$ is a key metabolite as it participates in a vast number of redox reactions. Over the past decades it gained additional attention as it is involved in many signalling reactions that play a role in cell regulation. Changes in $NAD^+$ metabolism have been found during aging and in metabolic disease such as diabetes. $NAD^+$ depending signalling reactions include the consumption of $NAD^+$ and require a constant replenishment of cellular $NAD^+$ pools. This can be done either by salvaging the product of these reaction, nicotinamide (Nam), or by synthesising $NAD^+$ *de novo* from the amino acids aspartate or tryptophan. As a systematic analysis of NAD metabolism has not been performed before, we first analysed the underlying metabolic network using elementary flux mode (EFM) analysis. As

$NAD^+$ can be synthesised and degraded by multiple routes, EFMs help to decompose the network and identify possible metabolic routes and analyse the effect of enzyme deletions. As human and yeast are by far best investigated organisms regarding NAD metabolism, but show remarkable differences in their NAD related enzyme composition, we initially reconstructed the NAD metabolism of these two species as a basis for our EFM-analysis [dFGZS11].

The results from this initial study showed that the vast majority of $NAD^+$ biosynthesising enzymes are present in yeast and human. However, the number of $NAD^+$-consuming enzymes differs substantially between the two species and there are some routes that are present in either human or yeast. This differences in the NAD metabolism called for the reconstruction of the evolutionary history of NAD metabolism. Previous studies for prokaryotes have shown that neither *de-novo* synthesis nor salvage of $NAD^+$ are universal and occur via modules of different genes [GSC+09]. However little is known on how the complexity of NAD metabolism evolved over time in higher organisms. Therefore, we have analysed the phylogenetic distribution of NAD metabolism related enzymes in 45, mainly higher eukaryotic species [GZP+12].

## 2 Major findings

We first reconstructed a metabolic network of $NAD^+$ biosynthesis in human and yeast. These were combinde to create a generalised model that comprises 113 EFMs. These are metabolic routes that are stoichiometrically and thermodynamically balanced and consists of a minimal set of enzymes that can operate at steady state. 50 EFMs can be found in human and 100 in yeast but only 40 are shared. Several of these EFMs constitute futile cycles, which are routes in the metabolic networks with no net transformation except hydrolysis of ATP. Whether these futile cycles are of physiological relevance depends on corresponding kinetics and regulation mechanisms. The much larger number of possible routes found in yeast is rather surprising. Another tendencies is that within the human network amidated forms are preferred, while yeast preferentially uses deamidated forms. Moreover, in both species elementary modes were identified that allow $NAD^+$ dependent signalling without net consumption of $NAD^+$. This was not known so far. Furthermore, Nam-mononucleotide adenelyl transferase (NMNAT) was identified to be essential for $NAD^+$ biosynthesis. This is consistent with experimental findings.

The combined human-yeast model was used as a basis for the phylogenetic analysis of $NAD^+$ metabolism in eukaryotes. Looking across 45 mainly eukaryotic species it becomes apparent that all investigated species are able to synthesise $NAD^+$ from at least one precursor and most species have more than one $NAD^+$ biosynthetic pathway. Some species lack the possibility to synthesise $NAD^+$ *de-novo* from aspartate or tryptophan and must therefore live under conditions which provide a sufficient amount of the NAD precursors nicotinic acid and Nam, commonly known as the vitamin niacin. The enzymes NADK and NMNAT are found in all species and can be considered to be essential for NAD metabolism supporting our results from the EFM analysis. Furthermore, the Preiss-Handler pathway is the most predominant NAD biosynthetic route among organisms suggesting a universal role for the generation of $NAD^+$.

The comparison between yeast and human showed that NamPT is an enzyme which can be found in humans but not in yeast, while the enzyme Nam-deaminase (NADA) is present in yeast but not in human. Both enzyme use Nam and provide the first step for Nam recycling to $NAD^+$ in the respective species. However, NamPT clearly provides a more efficient and economic route to $NAD^+$. It had been speculated that those two enzymes are mutually exclusive [RAGL03]. Surprisingly, the multispecies comparison reveals a scattered distribution of both enzymes across the animal kingdom. It rather suggests that NamPT enzymatic function got lost several times during evolution while the loss of NADA happened once and is common to all vertebrates. Interestingly, all species identified so far that have both NADA and NamPT have aquatic habitats. Whether, this has any physiological implications we do not know and we also do not know whether the enzymes are indeed expressed simultaneously.

Looking at the relation between $NAD^+$-consuming and Nam-recycling enzymes we initially assumed that increase of $NAD^+$-consuming enzymes should be reflected in an increase in biosynthetic routes. This is surprisingly not the case. In contrast we found a parallel phylogenetic appearance of the enzyme Nam-N-methyltransferase which is marking Nam, the product of $NAD^+$-consuming reactions, for further degradation and thus removing Nam from recycling to $NAD^+$.

## 3 Conclusions and future perspectives

The decomposition of the NAD metabolic network has revealed unexpected fluxes including futile cycles and $NAD^+$ signalling without net

consumption of $NAD^+$. The physiological relevance has to be shown experimentally. Furthermore, some reactions might not occur in the cell, as our models currently neglect compartmentalisation. The investigation and integration of the subcellular localisation and the identification of metabolite transporters is therefore crucial to understand NAD metabolism. As furthermore not all routes identified will be feasible under physiological conditions we are currently building a kinetic model to better understand the kinetic constraints that limit $NAD^+$-biosynthesis and -consumption.

The results from our phylogenetic analysis have revealed several interesting aspects that raise important issues. For example, why do some organisms encode both NADA and NamPT while many others do not? It has been suggested that the lack of NADA in vertebrates is compensated by gut microbiotic flora [GSC+09]. Therefore such an interplay between organisms could serve as a pool for the entry metabolite of the Preiss Handler pathway. It could also provide a possible explanation for the concurrent existence of NADA and NamPT in some species.

Another question arising from our analysis is, why higher eukaryotes require an enzyme for Nam-degradation whereas species with a low $NAD^+$-consumption do not? Again the answer might be provided by kinetic modelling as Nam is known to be a potent inhibitor of some $NAD^+$-consuming enzymes and might therefore interfere with $NAD^+$-dependent signalling.

## References


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