A close look at NAD biosynthesis

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Two new studies on the structure of an enzyme involved the synthesis of mammalian NAD shed new light on the evolutionary and biochemical complexity of this fundamental metabolic pathway.

NAD is one of the 'oldest' molecules in the history of biochemistry (**Fig. 1a**). Its discovery and biochemical characterization go back to the first half of the twentieth century. In the past several years, there has been a sort of renaissance in the biochemistry of NAD, with the discovery that in addition to its well-established role in redox biochemistry and energetic metabolism, nicotinamide cofactors can function as signaling molecules in a variety of cellular processes[1]. NAD is the substrate in mono- and poly-ADP ribosylation reactions that lead to the covalent modification of proteins. Likewise, NAD is the substrate of sirtuins, enzymes that catalyze the deacetylation of histones and other proteins. In addition, NAD is the precursor of signaling molecules such as cyclic ADP-ribose. All these reactions potentially lead to a progressive depletion of the intracellular levels of NAD, and there is therefore a continuous demand for NAD biosynthesis to maintain NAD homeostasis[2]. Interest in the pathways for NAD biosynthesis has been further augmented by the discovery of a link between the activity of the biosynthetic enzymes and lifespan[3]. On pages 582 and 661 of this issue, Kahn *et al.*[4] and Wang *et al.*[5] independently report on the structural characterization of mammalian nicotinamide phosphoribosyltransferase (Nampt), a central enzyme in NAD biosynthesis (**Fig. 1b**). Comparison with the structures of other phosphoribosyltransferases[6–8] reveals various unexpected features that help to explain the substrate specificity of mammalian Nampt, which specifically acts on nicotinamide and not on its derivatives or precursors (**Fig. 1**). These features have implications for understanding the molecular evolution of the phosphoribosyltransferase class of enzymes. Furthermore, the analysis of an enzyme-inhibitor complex[4] opens the way to inhibitor-design studies.

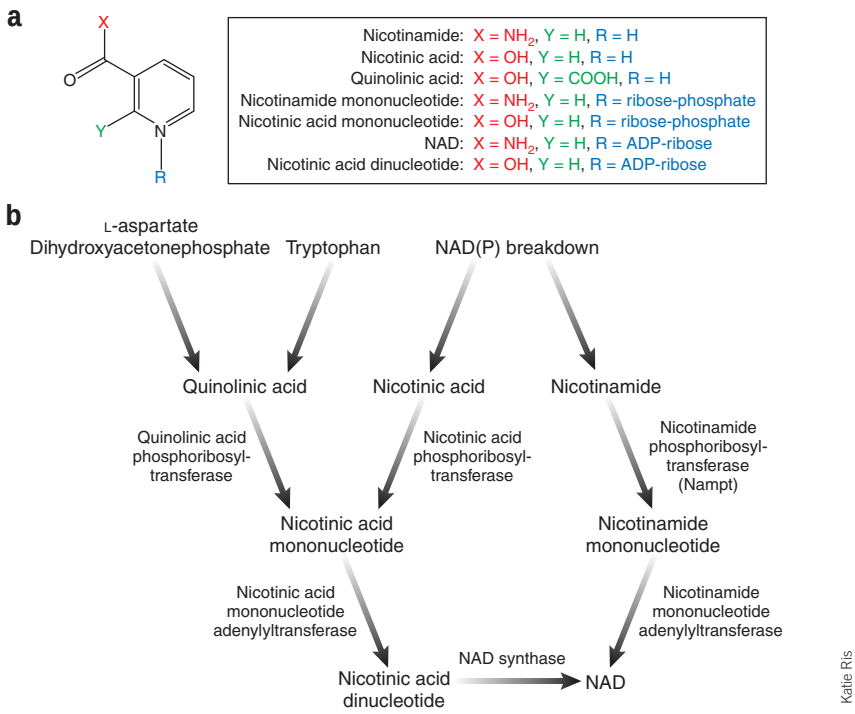

Figure 1 NAD and its biosynthesis (**a**) Chemical formula of NAD and its derivatives. (**b**) Simplified schematic view of the reactions involved in NAD biosynthesis, with emphasis on the reactions described in the text.

Depending on the organism, three different building blocks (quinolinic acid, nicotinic acid and nicotinamide) can be used for the biosynthesis of NAD (**Fig. 1**). Quinolinic acid is the precursor for the so-called *de novo* pathway; in eukaryotes, it is normally synthesized starting from tryptophan, whereas in bacteria it is synthesized from L-aspartate and dihydroxyacetonephosphate. In the so-called salvage pathways, NAD is synthesized starting from nicotinic acid and nicotinamide that are produced by the various reactions that degrade and consume NAD (**Fig. 1**). Quinolinic acid, nicotinic acid and nicotinamide are used by phosphoribosyltransferases to produce nicotinic acid mononucleotide or nicotinamide mononucleotide[6–8]. These molecules are then converted to the corresponding dinucleotides through adenylation reactions catalyzed by mononucleotide adenylyltransfer-

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ases[9–12]. Finally, NAD synthase catalyzes the amidation of nicotinic acid adenine dinucleotide to generate the NAD molecule. Recent years have witnessed tremendous advances in the structural biology of NAD biosynthesis, to the point that the three-dimensional structures of most of the enzymes involved in the pathway are known[13]. One of the most fascinating results revealed by these structural and biochemical investigations concerns the diversity in the specificities of the enzyme reactions. There are specific phosphoribosyltransferases for each of the precursors (**Fig. 1b**), the mammalian Nampt described by Kahn *et al.*[4] and Wang *et al.*[5] being strictly specific for nicotinamide. Likewise, adenylyltransferases differ in specificity depending on the organism; the *Escherichia coli* enzyme preferentially acts on nicotinic acid mononucleotide, whereas the mammalian and archaeal proteins are less stringent, acting on both nicotinamide and nicotinic acid mononucleotides[9–12].

Enzymes of the phosphoribosyltransferase class catalyze the addition of the phosphoribosyl moiety derived from phosphoribosylpyrophosphate to their specific substrates. The structures of bacterial quinolinic acid and nicotinic acid phosphoribosyltransferases have been described before[6–8], and with the structures reported in this issue of the mammalian Nampt[4,5], it has become possible to compare the phosphorib osyltransferases involved in bacterial and/or mammalian NAD biosynthesis. Although the three enzymes share a similar overall folding topology and quaternary structure, they differ substantially in the details of their architecture and active site geometry, implying that, despite their similar enzymatic activities, they have diverged considerably in the course of evolution. Of particular interest is the comparison of the active sites. Nampt is characterized by the presence of an aspartate residue that interacts with the nitrogen atom of the carbamide group of the nicotinamide substrate. In nicotinic acid phosphoribosyltransferase, this aspartate is replaced by a serine, suggesting that it is one of the crucial elements that dictates the specificity for either nicotinamide or nicotinic acid in these enzymes (**Fig. 1b**). Indeed, as shown by Khan *et al.*[4], the Asp→Ser mutation confers a dual specificity to the mutated Nampt, which is able to act on both nicotinamide and nicotinic acid. In this regard, it will be of interest to look at the structure of the mutant enzyme in complex with nicotinic acid, to see how the mutation enables the protein to bind in a catalytically competent orientation.

However, the story is even more complicated. A 10-residue insertion in an active site loop of Nampt induces a shift of a β-strand that modifies the shape of the active site cleft. This structural feature is another element that contributes to the specificity of mammalian Nampt. Thus, even a simple variation in substrate specificity such as the discrimination between a carbamide and a carboxylate substituent can be associated with rather elaborate changes in the conformation of the substrate-recognition site. This notion is further illustrated by the various adenylyltransferase enzymes involved in the NAD biosynthetic pathways[9–12]. Comparison between the bacterial nicotinic acid adenylyltransferase and the mammalian nicotinamide/nicotinic acid adenylyltransferase reveals changes in both the overall structure and the conformation of the active site residues. Thus, for the phosphoribosyltransferases as well as the adenylyltransferases, differences in substrate specificity seem to result from the additive effects of subtle alterations in the active site conformation and geometry, including the presence of ordered waters that mediate the interactions between the substrate and the protein.

NAD biosynthesis is emerging as one of the most thoroughly investigated metabolic pathways from both a biochemical and a structural standpoint[1,13]. Its characteristic feature is that it does not consist of a single defined pathway conserved in all organisms. The 'logic' and sequence of reactions are generally conserved, but depending on the organism, NAD biosynthesis starts from different precursors and takes different routes for the recycling of the products of NAD breakdown. Consequently, the NAD biosynthetic enzymes have acquired different specificities in the different organisms. The main theme emerging from comparative analysis of the structures of these enzymes is that of unexpected complexity. Changes in substrate specificity underlie a substantial degree of evolutionary divergence that results in large changes in the overall protein structure as well as in the conformation and geometry of the active site. These features should make the NAD biosynthetic enzymes ideal systems for *in vitro* evolution studies.

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The Pol II initiation complex: finding a place to start

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Yeast RNA polymerase II has been proposed to 'scan' template DNA for transcription start sites. A new study mapping promoter DNA trajectory through the preinitiation complex suggests a mechanism for how this occurs.

Over the past six years, a series of papers have provided high-resolution structures of yeast RNA polymerase II (Pol II) alone[1–4] and in elongation complexes with DNA and RNA[5,6]. Recently, a 2.3-Å-resolution image of a transcribing Pol II complex was solved, offering remarkable insight into transcription mechanism[7]. Missing from the picture, however, is the trajectory of DNA through the Pol II preinitiation complex (PIC), leaving us an incomplete structural image of transcription initiation. On page 603 of this issue, Miller and Hahn address this by mapping the path of promoter DNA through a functional PIC using DNA-tethered cleavage probes[8]. Their results suggest a mechanism for how yeast Pol II scans DNA for transcription start sites.

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