The Key Role of Nicotinamide Phosphoribosyltransferase in Nicotinamide Adenine Dinucleotide Metabolism

Nicotinamide adenine dinucleotide (NAD) represents an essential coenzyme whose role in cellular metabolism has been recognized for over a century (1). Despite this well-established role in cellular redox reactions, the precise identification of all biosynthetic routes leading to NAD has only been achieved lately. Several natural NAD precursors (tryptophan, nicotinic acid, nicotinamide, and nicotinamide riboside) have been identified in eukaryotes, with distinct roles according to organism and/or tissue origin (Fig. 1). Although biochemical and physiological data established nicotinamide as the most important and widely available NAD precursor in mammals, the gene (Nampt) that encodes nicotinamide phosphoribosyltransferase, which is the enzyme catalyzing the first and limiting reaction allowing NAD synthesis from this particular precursor, was only identified less than a decade ago (2). Quite unexpectedly for an enzyme that had all the “housekeeping” gene credentials, its identification derived from studies whose primary goal was to identify activation-induced or tissue-specific genes. In several independent studies, the expression of Nampt was found to be upregulated in immune cells upon activation (3–5), whereas another report identified this protein as a gene strongly expressed in adipocytes (6). It is also noteworthy that before the precise identification of its functional properties, Nampt was found to be overexpressed in tumor cells (7). The different names given to this protein (pre-B-cell colony enhancing factor, visfatin, and Nampt) reflect the distinct roles that it may exert depending on tissue origin and/or physiological context.

It is unlikely that the wide range of Nampt expression levels reported in the literature represent a biological response to match an increase in energetic demand. Indeed, during redox reactions (such as ATP biosynthesis), NAD is rapidly interconverted between its oxidized and reduced forms without net consumption. Noteworthy, nondividing, innate immune cells such as macrophages and dendritic cells have been found to express high levels of this protein, suggesting alternative roles for intracellular NAD in these cells (8). The discovery of several enzymes able to use NAD as a substrate, rather than a cofactor, has shed a novel light on the possible relationship between NAD biosynthesis and the regulation of cellular responses (9, 10). A common trait of these reactions is the use of NAD as a donor of ADP-ribose, leading to the net consumption of NAD and the concomitant release of nicotinamide. These observations suggest that Nampt-dependent recycling of nicotinamide to NAD (see Fig. 1) may represent a physiologically important homeostatic mechanism to avoid depletion of the intracellular NAD pool during its active use as a substrate. Based on these considerations, it is tempting to assume that increased levels of intracellular Nampt reflect the increased activity of several NAD-degrading enzymes, thus providing a novel molecular link between NAD metabolism and the regulation of several aspects of cell physiology. In line with this hypothesis, Nampt activity has been shown to enhance cellular proliferation (11) and to tip the balance toward cell survival following a genotoxic insult (12). Also, recent findings showed that Nampt plays a central role in controlling the circadian clock machinery, by dictating the periodical oscillations of some of its key transcription factors (13). At the immune level, Nampt has been shown to promote myeloid (14) and lymphoid (15) differentiation and to increase specific cytokine production (8). In many cases, an intermediate NAD
NAD Biosynthesis and Resistance to Cellular Stress

Poly(ADP-ribosylation) mediated by the abundant nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) represents a well-characterized immediate response to genotoxic damage induced by oxidative stress, alkylating agents, or ionizing radiations (10). PARP-1 binds to DNA strand breaks and catalyzes the transfer of successive units of the NAD-derived ADP-ribose to several nuclear proteins, including PARP-1 itself. This post-translational modification has been shown to facilitate DNA repair and plays, therefore, a protective role in response to moderate genotoxic stress. Uncontrolled PARP-1 activity in response to high levels of DNA damage can, however, lead to a severe depletion of intracellular NAD pools, decreased resistance to stress, and cell death (10). This dual role of PARP-1 (DNA repair following mild DNA damage and cell death in response to excessive genotoxic insult) probably represents a fail-safe mechanism for limiting the accumulation of cells containing heritable genetic errors. Cell death in response to PARP overactivation is mediated by several molecular mechanisms, including the depletion of intracellular ATP levels, reduced mitochondrial function, release of pro-apoptotic mitochondrial factors, and reduced activity of prosurvival factors (10). Because nicotinamide represents the natural end product of enzymatic reactions catalyzed by PARP family members and is largely available intracellularly, an increase in Nampt activity appears as a physiological response to replenish cellular NAD levels in cells exposed to stress. In agreement with this hypothesis, we and others have recently shown an important role for the Nampt enzymatic activity in regulating cellular sensitivity to PARP-mediated stress. In particular, cells expressing reduced levels of Nampt or exposed to a potent Nampt pharmacological inhibitor (APO866; ref. 16) displayed...
an increased sensitivity to cell death induced by PARP-activating agents (15). Conversely, overexpression of a catalytically active Nampt increased intracellular NAD levels and led to partial cell resistance to genotoxic insult. Based on these observations, it is tempting to conclude that elevated levels of Nampt may in fact represent a physiological adaptive response increasing cell resistance to environmental stress.

**Intracellular NAD Levels, Sirtuins, and the Regulation of Tumor Necrosis Factor Synthesis**

The elevated expression of Nampt in cells of the innate immune system (such as macrophages and dendritic cells) prompted us to evaluate a possible link between intracellular NAD levels and inflammation. Taking advantage of the Nampt inhibitor APO866, we have been able to establish a functional link between intracellular NAD levels and the secretion of several cytokines in response to microbial products (8). Reduction of intracellular NAD levels led to a decreased production of several pro-inflammatory cytokines [in particular tumor necrosis factor (TNF)] in activated macrophages or dendritic cells. This inhibitory effect was not related to a metabolic defect, because cells displaying low intracellular NAD levels retained the ability to secrete other proteins (such as the chemokine RANTES) in response to the same stimuli. Rather, these findings suggest a role for a NAD-dependent biological event in the control of selected cytokine synthesis. Numerous experimental observations have identified sirtuins, a family of seven NAD-dependent deacetylases in mammals (SIRT1 to SIRT7) as sensitive sensors of intracellular NAD levels (8, 17). A pharmacological approach using several structurally unrelated sirtuin inhibitors confirmed that optimal secretion of TNF is under the control of a sirtuin member. A positive in vitro screen led us to identify SIRT6 as the NAD-dependent enzyme able to regulate TNF production by acting at a post-transcriptional step. These observations reveal therefore an unsuspected link between NAD metabolism and the control of an inflammatory response. Adequate intracellular NAD levels (under the major influence of Nampt) regulate SIRT6 activity, which in turn positively controls TNF mRNA translation.

**Nampt as a Possible Therapeutic Target**

Because of its central role in the recycling pathway allowing NAD biosynthesis from nicotinamide, Nampt occupies a pivotal position in controlling the activity of several NAD-dependent enzymes. This conclusion is supported by the observation that although NAD can in principle be synthesized by alternative sources, virtually all cells of the organism rely on nicotinamide to maintain adequate intracellular NAD levels (18), most probably because this precursor is continuously generated as a by-product of all NAD-consuming reactions characterized to date. Moreover, intracellular NAD concentrations seem to exceed the minimum levels required for survival and the accomplishment of routine cellular functions (16). The available data strongly suggest that cells adjust systemic NAD biosynthesis to regulate the activity of several NAD-dependent enzymes such as sirtuins. The recent finding that rhythmic oscillations in protein levels of Nampt lead to circadian oscillation on NAD strongly support the central role of Nampt in the regulation of NAD homeostasis (13).

The emerging picture arising from these studies indicates an important functional link between systemic NAD biosynthesis and cell physiology through a process referred to as "chemomodulation" (9), whereby levels of intracellular NAD (substrate) and nicotinamide (acting as an endogenous end product inhibitor) affect the enzymatic activity of sirtuins. The cellular responses that seem to be under the influence of NAD are very diverse, probably reflecting the large array of sirtuin substrates and their role in numerous regulatory pathways. This complex regulatory network should in principle disqualify Nampt as a potential target for pharmacological intervention. Indeed, reduced NAD levels should affect the activity of several sirtuin members, causing multiple, and possibly unrelated, biological effects in vivo. It is, however, interesting to note that the evidence accumulated to date suggests a possible therapeutic window for Nampt inhibitors in the field of inflammation and cancer. Long term in vivo administration of APO866 was able to effectively control the development of an in vivo inflammatory response (notably in an animal model of sustained inflammatory response such as collagen-induced arthritis; ref. 19), without overt toxicity. Similarly, the same compound displayed potent antitumor activity in vivo against hematological malignancies (20). Taken together, these studies indicate a possible specificity of Nampt inhibitors in the control of immune cell viability and function. Although the notion that an inhibitor targeting such a central metabolic pathway (NAD biosynthesis) may display some level of specificity in vivo may be counterintuitive, it is interesting to consider that: (i) in contrast to other organs (such as the liver and the kidney) immune cells, with the possible exception of monocytes, seem to rely exclusively on the Nampt salvage pathway for NAD biosynthesis (15); (ii) actively proliferating cells (such as tumors and proliferating immune cells) display increased PARP activity, rendering these cells highly dependent upon Nampt activity for adequate survival (21); (iii) optimal secretion of pro-inflammatory cytokines such as TNF is under the control of sirtuins (8); and finally (iv) sirtuins play an important role in regulating cell survival following genotoxic stress (12).

Two seemingly unrelated effects may therefore concur to render tumor cell growth dependent upon adequate intracellular NAD concentrations. As previously shown, high intracellular NAD levels may act through sirtuins to favor cell survival, especially in response to genotoxic insult. It is noteworthy that p53 has been identified as a target of SIRT1. It has been suggested that by linking the acetylation status of p53 to intracellular NAD levels, Nampt activity may protect cells from premature senescence (22). In agreement with these findings, sirtuin inhibitors have been successfully used...
in vivo to downregulate tumor cell growth (23). Moreover, and although its mode of action is still a matter of debate, TNF has been recently recognized as a potential tumor promoting factor (24). Whether produced by the tumor itself or by infiltrating immune cells, TNF signaling in the tumor microenvironment is likely to stimulate tumor growth, and strategies to neutralize TNF activity have provided some levels of protection in both animal and clinical studies. In conclusion, Nampt activity may play a dual role in tumor development, by promoting both cells survival and TNF secretion. Therefore, by targeting these two unrelated biological pathways controlling the development of tumors in vivo, Nampt inhibitors may represent novel and promising antitumor chemotherapeutic agents.

References

7. Van Beijnum JR, Moerkerk PT, Gerbers AJ, et al. Target validation for pathways controlling the development of tumors in vivo, Nampt activity may play a dual role in tumor

Disclosure of Potential Conflicts of Interests

Topo Target Denmark: partial financial support for this project. M. Galli, F. Van Gool, and O. Leo patent applications to the World Intellectual Property Organization (WIPO) pertaining to the possible use of APO866 to treat inflammatory-related disorders.

Grant Support

The Belgian Program in Interuniversity Poles of Attraction, the Fonds National de la Recherche Scientifique de Belgique, and the Région Wallonne.

Received 7/2/09; revised 9/24/09; accepted 9/24/09; published OnlineFirst 12/22/09.