

Discovery, metabolism and functions of NAD and NADP

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Nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) are two major players in metabolism as they participate as electron carriers in a multitude of redox reactions. Moreover, they act in life and death decisions on a cellular level in all known life forms. NAD and NADP both exist in two states; the oxidized forms are characterized by a positive charge on the nicotinamide (Nam) moiety, denoted NAD^+ and NADP^+ respectively. The reduced forms are denoted NADH and NADPH (Figure 1).

The independent discoveries of NAD(P) as vitamins and co-enzymes

Vitamin B_3 is a collective term for the two NAD(P) precursors nicotinic acid (NA) (also referred to as niacin) and Nam, as well as their corresponding ribosides. Niacin and Nam were found to be essential nutrients after severe outbreaks of pellagra (originally thought to be a new pestilence) in 18th Century Europe. Large outbreaks of pellagra occurred in North America in the early 20th Century. Pellagra (from Italian: *pelle* = skin; *agra* = sour) is characterized by dermatitis, diarrhoea, dementia and ultimately death, and was widespread in the hundreds of thousands of poor people living on a diet mostly composed of corn or maize flour. Although successfully consumed by American Indians for centuries, degerminated maize does not contain niacin in a bioavailable form. The crucial difference was that the American Indians traditionally cooked whole grain

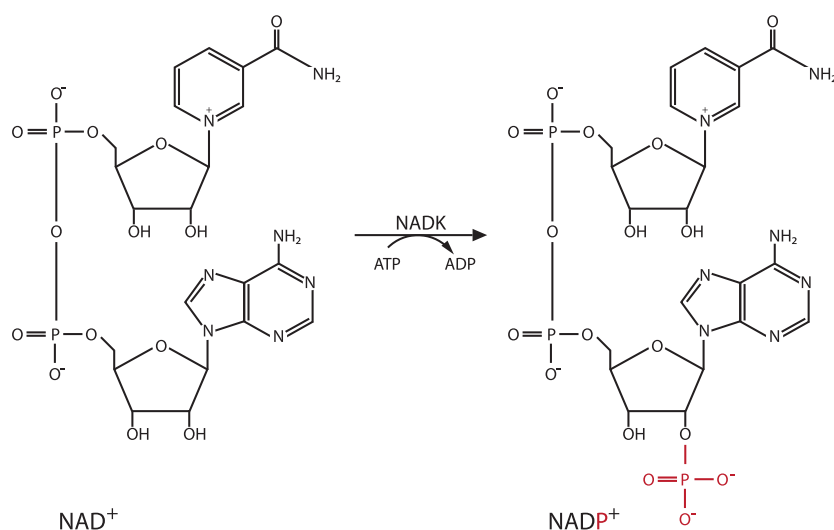


Figure 1. Structures of NAD^+ and NADP^+ and the reaction catalysed by NAD kinase (NADK). The phosphate group is shown in red.

Abbreviations: AIF, apoptosis-inducing factor; NA, nicotinic acid; NAADP, nicotinic acid–adenine dinucleotide phosphate; NADA, nicotinamidase; NADS, NAD synthetase; Nam, nicotinamide; NAMN, nicotinic acid mononucleotide; NMNAT, NMN adenylyltransferase; NamPRT, nicotinamide phosphoribosyltransferase; NRK, nicotinamide riboside kinases; OAADPR, *O*-acetyl-ADP-ribose; PARP1, poly(ADP-ribose) polymerase 1; PRPP, phosphoribosyl pyrophosphate; QA, quinolinic acid; Sir2, silent information regulator-2; TCA, tricarboxylic acid

Key words: pellagra, Preiss–Handler pathway, salvage pathway, signal transduction, vitamin B_3

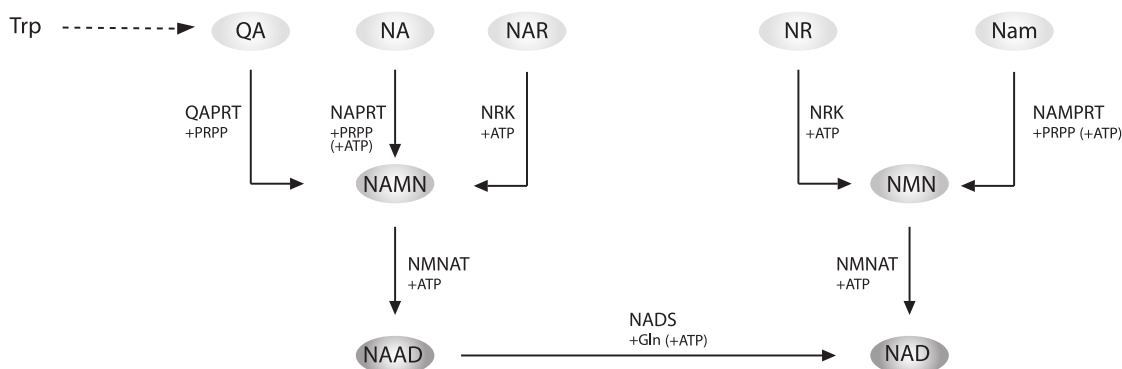


Figure 2. Overview of NAD biosynthesis. The different entry points use precursors containing the pyridine moiety. QA and NA are converted into nicotinic acid mononucleotide (NAMN) by their respective phosphoribosyltransferases (QAPRT and NAPRT). Similarly, nicotinamide phosphoribosyltransferase (NamPRT) yields NMN from Nam. The riboside forms of Nam and NA (NR and NAR respectively) may also serve as precursors for NMN and NAMN, these reactions being catalysed by nicotinamide riboside kinases (NRKs). Formation of the dinucleotide (NAD or NAAD) is catalysed by nicotinamide mononucleotide adenylyltransferases (NMNATs). NAAD is amidated to NAD by NAD synthetase (NADS).

maize with limewater (or wood ashes) in a process called nixtamalization giving a product they called hominy. This alkaline treatment rendered the carbohydrate-bound niacin available for uptake in the digestive tract. When maize was imported to Europe, this practice was not imported with it and so the essential nutrient was lost, leading to the outbreaks of pellagra.

After some rather unsavoury experiments conducted by Joseph Goldberger, for instance the infamous Rankin State Prison Farm experiment in 1915, it was finally concluded in 1917 that pellagra was induced by a poor diet, a hypothesis that had been long held by Italian physiologists. Ironically, Casimir Funk, searching for a treatment for beriberi, later known to be caused by a deficiency in thiamine (vitamin B₁), identified NA in yeast as early as in 1913, but discarded it as a vitamin since it had no anti-beriberi effect¹. After a long search for the pellagra preventive factor, Conrad Elvehjem discovered in 1937 that NA and Nam could cure black tongue disease, the canine version of pellagra. Subsequently, the supplementation of ground meal with niacin resulted in a near eradication of pellagra in the developed world¹.

Independently of the quest to identify the pellagra preventive factor, NAD was found to act as an essential cofactor in metabolism. Indeed, NAD was the first organic cofactor to be identified when Arthur Harden and William John Young found NAD⁺ to be a heat-stable factor for boosting alcohol fermentation in yeast in 1906. Chemically identified as a nucleotide in 1930 by Hans von Euler-Chelpin, NAD⁺ was shown to be involved in redox reactions by Otto Warburg in 1936. In 1950, Arthur Kornberg was the first to show the direct generation of NADP⁺ from NAD⁺ using partially purified NAD kinase from yeast extracts. In the following years, the

importance of NAD(P) as energy transmitters in both catabolism and anabolism was fully appreciated. Only decades later was the critical involvement of NAD(P) in a variety of signalling pathways unravelled. Both NAD⁺ and NADP⁺ are degraded in signalling reactions, either in protein modifications such as ADP-ribosylation and deacetylation or to form messenger molecules involved in calcium signalling. The existence of these ‘consumption’ processes indicates why continuous biosynthesis of NAD(P) is essential.

Biosynthesis of NAD(P)

NAD, and subsequently NADP, can originate from several routes depending on the organism and the precursors used (Figure 2). *De novo* biosynthesis in humans, yeast and some bacteria involves the conversion of tryptophan into quinolinic acid (QA), which in turn can be condensed with phosphoribosyl pyrophosphate (PRPP) to yield nicotinic acid mononucleotide (NAMN). As an alternative to tryptophan, some plants and other bacteria use aspartate as a precursor for the synthesis of QA. In the classical Preiss–Handler pathway, NA is converted into NAMN by the corresponding phosphoribosyltransferase, NAPRT. NAD can also originate from the recycling of its own degradation product, namely Nam. This is known as the salvage pathway. The first step of the salvage pathway, catalysed by nicotinamide phosphoribosyltransferase (NamPRT), is the generation of nicotinamide mononucleotide (NMN) by condensation with phosphoribosyl pyrophosphate. Furthermore, the riboside forms of NA and Nam seem to be widespread intermediates of NAD metabolism. They can be phosphorylated by nicotinamide riboside kinases (NRKs) to the corresponding mononucleotides (NAMN and NMN). The merging point of all pathways is the

reversible transfer of the adenylyl group of ATP on to the mononucleotide. This reaction is catalysed by NMN adenylyltransferases (NMNATs) for which three human isoforms have been identified. These three isoforms display different subcellular localizations and hence indicate compartmentalization of NAD synthesis. If the precursor used to synthesize NAD is in the acid form, the last step to NAD synthesis consists of the conversion to the amide form by NAD synthetase (NADS) which, in humans, is the only known transition between the acid and the amide. However, many organisms such as bacteria, yeast, plants and some invertebrates express a nicotinamidase (NADA), an enzyme that catalyses the deamidation of Nam to NA. In most of these organisms this step is essential to use Nam as NAD precursor, because they lack NamPRT activity². That is the deamidation of Nam allows them to use the Preiss–Handler pathway for the recycling of the NAD precursor.

Finally, the generation of NADP is mediated by NAD kinases (NADKs) that transfer a phosphate group from ATP on to the 2'-hydroxyl group of the adenosine ribose moiety (Figure 1). Remarkably, at least one gene encoding NADK appears to be present in all organisms studied in this regard, except for a few intracellular parasites such as *Chlamydia trachomatis*, which appear to use the NADP generated by the host cells.

Functions of NAD and NADP in metabolism

The electron-transfer properties of NAD and NADP and their role as major cofactors in redox reactions have been widely studied and described. Many dehydrogenases use these molecules to serve as major electron acceptors or donors and almost every metabolic pathway requires them. The pairs NAD⁺/NADH and NADP⁺/NADPH have similar redox potentials, and many dehydrogenases can use either coenzyme. NADK is present in virtually all organisms, showing that both NAD and NADP are essential and that they have adopted specific roles throughout evolution. Within cells, NAD is predominantly present in the oxidized form and its concentration is in the low millimolar range. The pyridine nucleotides drew a lot of attention following their identification as electron acceptors for a variety of reactions catalysed by dehydrogenases. For example, NAD is involved in catabolic reactions including glycolysis and the tricarboxylic acid (TCA) cycle. NADP, on the other hand, is present at lower concentrations and, in contrast with NAD, is mostly reduced and serves as an electron donor for reductive biosyntheses. Furthermore, NADPH serves as a reducing agent to regenerate antioxidant systems such as thioredoxins and glutathione. In turn, to keep the cellular NADP in a reduced state, dedicated systems (e.g. glucose-6-phosphate dehydrogenase in the cytosol and

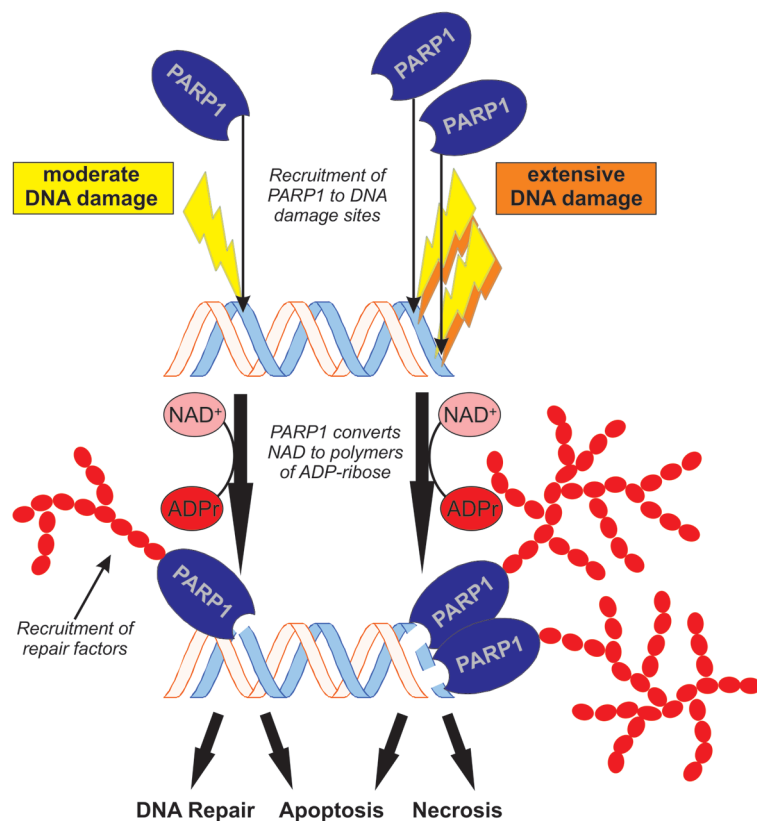


Figure 3. The role of PARP1 and NAD⁺ in DNA damage repair and cell death pathways. Upon DNA damage, PARP1 (blue) is activated and starts to generate long branched polymers of ADP-ribose (red) using NAD⁺ (pink) as substrate. The polymers can either be attached to PARP1 itself or to other target proteins such as transcription factors (not shown). Mild to moderate DNA damage leads to recruitment of DNA repair factors to the DNA damage site via the polymers, whereas moderate to extensive DNA damage leads to a higher consumption of NAD⁺ through massive polymer formation and subsequently to the initiation of cell death, either through apoptosis or necrosis.

isocitrate dehydrogenase 2 in the mitochondria) are up-regulated under conditions of oxidative assaults³.

Signalling functions of NAD(P)

An entirely different scope of functions of the pyridine nucleotides was more recently uncovered. Degradation of these dinucleotides plays a major role in signalling events with all these different reactions sharing a common feature: requirement for oxidized NAD(P) to be cleaved to nicotinamide and ADP-ribose (or 2'-phospho-ADP-ribose). Thus ADP-ribosylation is a post-translational modification that requires NAD⁺ as a substrate: NAD⁺ is cleaved to liberate nicotinamide and the ADP-ribose moiety is transferred on to target proteins. In the 1960s, the first NAD⁺-dependent protein modification was identified as poly(ADP-ribosyl)ation (PARylation) and about two decades later, mono-ADP-ribosylation was

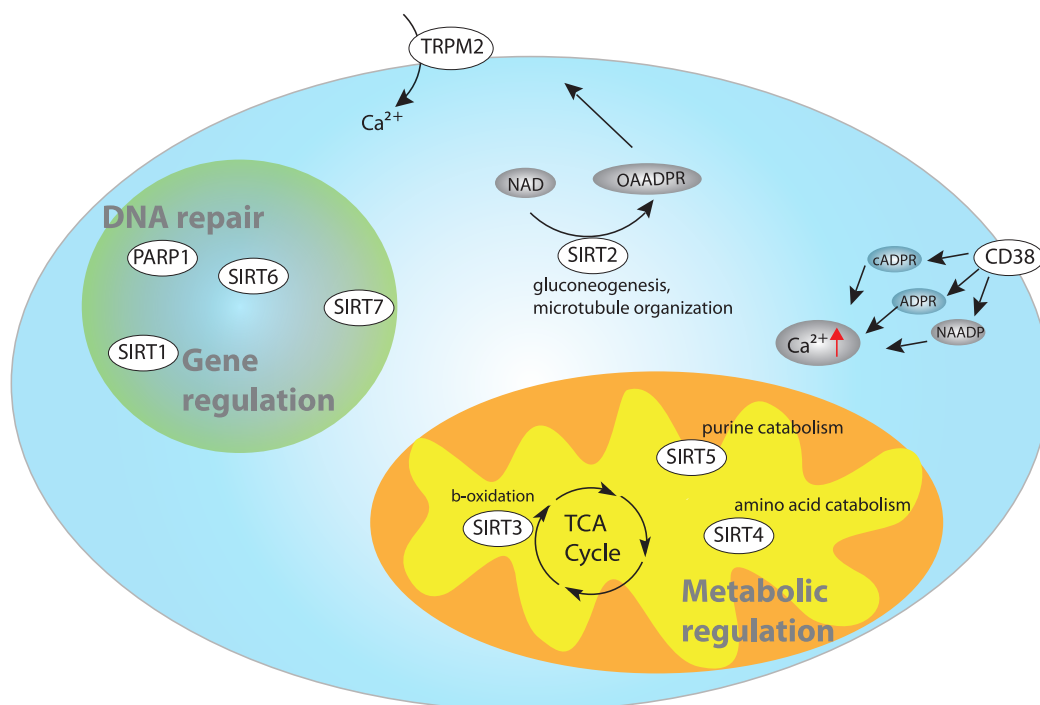


Figure 4. Examples of cellular signalling processes requiring NAD(P)⁺. NAD-dependent enzymes such as PARPs and sirtuins localize to different subcellular compartments. Nuclear PARP1 and SIRT6 play a role in DNA repair, whereas SIRT1 is an important factor in transcriptional regulation. SIRT3 is the major deacetylase in mitochondria and influences, among other processes, β -oxidation and the tricarboxylic acid (TCA) cycle. SIRT4 and SIRT5 contribute to other metabolic pathways such as amino acids and purine catabolism. Cytosolic SIRT2 is involved in gluconeogenesis and microtubule organization. *O*-acetyl-ADP-ribose, a product generated upon deacetylation by sirtuins can serve as messenger for calcium signalling by activating TRPM2 channels in the plasma membrane. CD38 is a multifunctional NAD(P)⁺ glycohydrolase capable of producing calcium mobilizing messengers from pyridine nucleotides, including cyclic ADP-ribose, ADP-ribose and NAADP.

uncovered as a catalytic activity of some bacterial toxins. Mono-ADP-ribosylation has now been established as an important process on the surface, primarily of immune cells. PARylation, attachment of ADP-ribose polymers on to their target proteins, plays a major role in DNA repair⁴ (Figure 3). Upon single strand breaks, poly(ADP-ribose) polymerase 1 (PARP1), is recruited to the damage site and catalyses PAR formation on proteins, PARP1 being a major acceptor itself. As a result, the modified transcription factors lose their affinity to the DNA, which in turn becomes more accessible for the repair machinery. Moreover, PAR is thought to mediate the recruitment of DNA repair enzymes. The release of mitochondrial apoptosis-inducing factor (AIF) can be a result of strong PAR accumulation and may thereby mediate apoptosis. If the extent of DNA damage happens to be too extensive, overactivation of PARP1 may also trigger necrosis, because of substantial loss of cellular NAD⁺ (Figure 3). PARP1 is a member of a superfamily comprising 17 members out of which four members have PARylation activities (PARP1 and 2, tankyrase 1 and 2) whereas most of the remaining members have mono-ADP-ribosylation activities.

The scope of signalling functions of pyridine nucleotides has been extended by the discovery that both NAD⁺ and NADP⁺ are important contributors to intracellular calcium signalling. Remarkably, derivatives of both NAD⁺ and NADP⁺ have been associated with these pathways. NAADP (nicotinic acid-adenine dinucleotide phosphate), resulting from the exchange of the Nam of NADP with NA by NAD glycohydrolases such as CD38, is considered to be the most potent intracellular calcium mobilizing agent⁵.

Of considerable current interest is the recently discovered role of NAD as a co-substrate for specific protein deacetylation. Initially observed in yeast, the silent information regulator-2 (Sir2) mediates, among other processes, histone deacetylation. As opposed to NAD-independent deacetylases, Sir-2 homologues (dubbed sirtuins) do not lead to the release of acetate. Instead, the mechanism involves a reaction between the acetyl-lysine and NAD⁺. As a result, Nam is cleaved from NAD⁺ and the acetyl group transferred on to the remaining ADP-ribose to form *O*-acetyl-ADP-ribose (OAADPR), which in turn can also serve as a messenger molecule. The seven

members of the mammalian sirtuin family differ by their subcellular localization and specificity for acyl groups' lengths. For example, SIRT1 activates peroxisome proliferator activated receptor γ co activator α (PGC1 α), an important transcription factor for the activation of mitochondrial genes and mitochondrial biogenesis. Similarly, SIRT1 deacetylates FOXO and thus plays a role in glucose and lipid metabolism. Cytosolic SIRT2 is involved, for example, in gluconeogenesis* (phosphoenol pyruvate carboxykinase 1) and microtubule organization (α -tubulin). The mitochondrial SIRT3 is also an important player in metabolic homeostasis. It is the only identified mitochondrial deacetylase and, as such, it regulates, among other processes, fatty acid oxidation (long-chain acyl-CoA dehydrogenase), production of ketone bodies (3-hydroxy-3-methylglutaryl-CoA synthase 2), and the TCA cycle (isocitrate dehydrogenase 2) and glutamate dehydrogenase. SIRT4 and SIRT5 are also mitochondrial, but, interestingly, they do not display strong deacetylation activities. Rather, SIRT4 has been described as a mono-ADP-ribosyltransferase (glutamate dehydrogenase), this activity being involved in amino acid catabolism, whereas SIRT5 is a deacylase, which favours the removal of more complex acyl groups such as succinyl and myristoyl groups. SIRT6 and SIRT7 have been localized to the nucleus, similar to SIRT1. SIRT6 has been described as mono-ADP-ribosyltransferase able to modify PARP1 and may thus be involved in DNA repair. So far, little is known about the targets and activity of SIRT7⁶.

All of these examples show that NAD and NADP can virtually influence every cellular pathway, making them cornerstones of cellular functions (Figure 4).

Potential of NAD(P) metabolism as a target for disease treatment

Although it is clear, as in the case of pellagra, that a shortage of NAD is harmful, it still remains to be seen whether high dosages of NAD precursors can be applicable as a therapeutic or as beneficial diet supplements. The supplementation with the NAD precursors nicotinamide riboside or NMN in mice has given promising results; for example, in an Alzheimer's disease model and as protection against the development of age- or diet-induced diabetes. These effects are likely to be mediated primarily by the sirtuins². There is an ongoing debate as to whether sirtuin activity can regulate longevity. Elevated sirtuin activity has been proposed to extend the lifespan of organisms such as yeast and *Caenorhabditis elegans*, more recently also in male mice^{7,8}. However, these effects appear to be indirect. Given the complexity of the signalling pathways involved in these processes, the exact mechanisms of how sirtuin activity could affect longevity are still to be clarified.

Another entry point for the therapeutic potential through manipulation of NAD levels is via inhibition of PARP1. Given the important role of PARP1 in the detection and repair of both single-stranded and double-stranded DNA breaks, its inhibition has been considered as a treatment to sensitize tumour cells to genotoxic stress (e.g. induced by radiotherapy or chemotherapy), especially cells that have deficiencies in DNA damage repair, such as a mutation in the *BRCA* genes. As such, PARP inhibitors are now in Phase III clinical trials for the treatment of *BRCA*-deficient tumour(s). In addition, the usage of NamPRT inhibition is potentially interesting in cancer treatment. Cancer cells have

* Targets involved in the different processes cited are mentioned in parentheses.

References

1. Sydenstricker, V.P. (1958) *Am. J. Clin. Nutr.* **6**, 409–414
2. Dölle, C., Skoge, R.H., Vanlinden, M.R. and Ziegler, M. (2013) *Curr. Top. Med. Chem.* **13**, 2907–2917
3. Agedal, L., Niere, M. and Ziegler, M. (2010) *Redox Rep.* **15**, 2–10
4. De Vos, M., Schreiber, V. and Dantzer, F. (2012) *Biochem. Pharmacol.* **84**, 137–146
5. Berger, F., Ramirez-Hernandez, M.H. and Ziegler, M. (2004) *Trends Biochem. Sci.* **29**, 111–118
6. Houtkooper, R.H., Pirinen, E. and Auwerx, J. (2012) *Nat. Rev. Mol. Cell Biol.* **13**, 225–238
7. Kanfi, Y., Naiman, S., Amir, G. et al. (2012) *Nature* **483**, 218–221
8. North, B.J., Rosenberg, M.A. and Jeganathan, K.B. (2014) *EMBO J.* **33**, 1438–1453
9. Li, M. and Yu, X. (2014) *Oncogene*, doi:10.1038/onc.2014.295
10. Chiarugi, A., Dölle, C., Felici, R. and Ziegler, M. (2012) *Nat. Rev. Cancer* **12**, 741–752



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