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REVIEW

The Regulatory Role of NAD in Human and Animal Cells

V. A. Kulikova[1,2], D. V. Gromyko[1], and A. A. Nikiforov[1,2,a]*

[1]*Institute of Cytology, Russian Academy of Sciences, 194064 St. Petersburg, Russia*
[2]*Peter the Great St. Petersburg Polytechnic University, 195251 St. Petersburg, Russia*
[a]*e-mail: andrey.nikiforov@gmail.com*

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Abstract—Nicotinamide adenine dinucleotide (NAD) and its phosphorylated form NADP are the major coenzymes in the redox reactions of various essential metabolic pathways. NAD^+ also serves as a substrate for several families of regulatory proteins, such as protein deacetylases (sirtuins), ADP-ribosyltransferases, and poly(ADP-ribose) polymerases, that control vital cell processes including gene expression, DNA repair, apoptosis, mitochondrial biogenesis, unfolded protein response, and many others. NAD^+ is also a precursor for calcium-mobilizing secondary messengers. Proper regulation of these NAD-dependent metabolic and signaling pathways depends on how efficiently cells can maintain their NAD levels. Generally, mammalian cells regulate their NAD supply through biosynthesis from the precursors delivered with the diet: nicotinamide and nicotinic acid (vitamin B3), as well as nicotinamide riboside and nicotinic acid riboside. Administration of NAD precursors has been demonstrated to restore NAD levels in tissues (i.e., to produce beneficial therapeutic effects) in preclinical models of various diseases, such as neurodegenerative disorders, obesity, diabetes, and metabolic syndrome.

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Nicotinamide adenine dinucleotide (NAD) and its phosphorylated form nicotinamide adenine dinucleotide phosphate (NADP) are co-enzymes in the redox reactions of key metabolic pathways involving reversible conversion of these dinucleotides from the oxidized ($NAD(P)^+$) to the reduced (NAD(P)H) form (Fig. 1). NAD^+ is also a substrate for several families of regulatory proteins controlling central signaling pathways in human and animal cells. Proteins responsible for NAD^+-dependent reactions of mono(ADP-ribosyl)ation, poly(ADP-ribosyl)ation, and protein deacetylation are ADP-ribosyltransferases, poly(ADP-ribose) polymerases, and protein deacetylases (sirtuins), respectively. These enzymes are involved in the regulation of such vitally important processes as gene expression, DNA repair, apoptosis, mitochondrial biogenesis, unfolded protein response, and others [1-5]. NAD^+ is a precursor for the synthesis of secondary messengers responsible for cellular calcium mobilization (Fig. 1) [6]. Because above mentioned NAD^+-dependent regulatory mechanisms are associated with the cleavage of nicotinamide from the dinucleotide, a certain level of NAD must be constantly maintained in the cell for these mechanisms to be efficient. Changes in the NAD content in cells and tissues are associated with a wide variety of diseases, such as neurodegenerative disorders, diabetes, obesity, metabolic syndrome, and cancer [7-9].

The major mechanism for the regulation of NAD levels in human and animal cells is its biosynthesis from precursors delivered through the diet. Tryptophan, pyridine bases nicotinamide (Nam) and nicotinic acid (NA) also known as vitamin B3, nicotinamide riboside (NR), and nicotinic acid riboside (NAR) are precursors for the intracellular NAD^+ biosynthesis. These precursors are used to generate nicotinamide mononucleotide (NMN) or nicotinic acid mononucleotide (NAMN), which are then converted into NAD^+ and nicotinic acid adenine

Abbreviations: ADPR, ADP-ribose; cADPR, cyclic ADP-ribose; AIF, apoptosis inducing factor; ARH, ADP-ribosylhydrolase; ART, ADP-ribosyltransferase; ARTC, clostridial toxin-like ADP-ribosyltransferase; ARTD, diphtheria toxin-like ADP-ribosyltransferase; ER, endoplasmic reticulum; KDAC, lysine deacetylase; NA, nicotinic acid; NAAD, nicotinic acid adenine dinucleotide; NAADP, nicotinic acid adenine dinucleotide phosphate; Nam, nicotinamide; NAMN, nicotinic acid mononucleotide; NAR, nicotinic acid riboside; NAPRT, nicotinic acid phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NMNAT, nicotinamide mononucleotide adenylyltransferase; NR, nicotinamide riboside; PARP, poly(ADP-ribose) polymerase; Sir2, silent information regulator 2; SIRT1-7, sirtuins 1-7.

* To whom correspondence should be addressed.

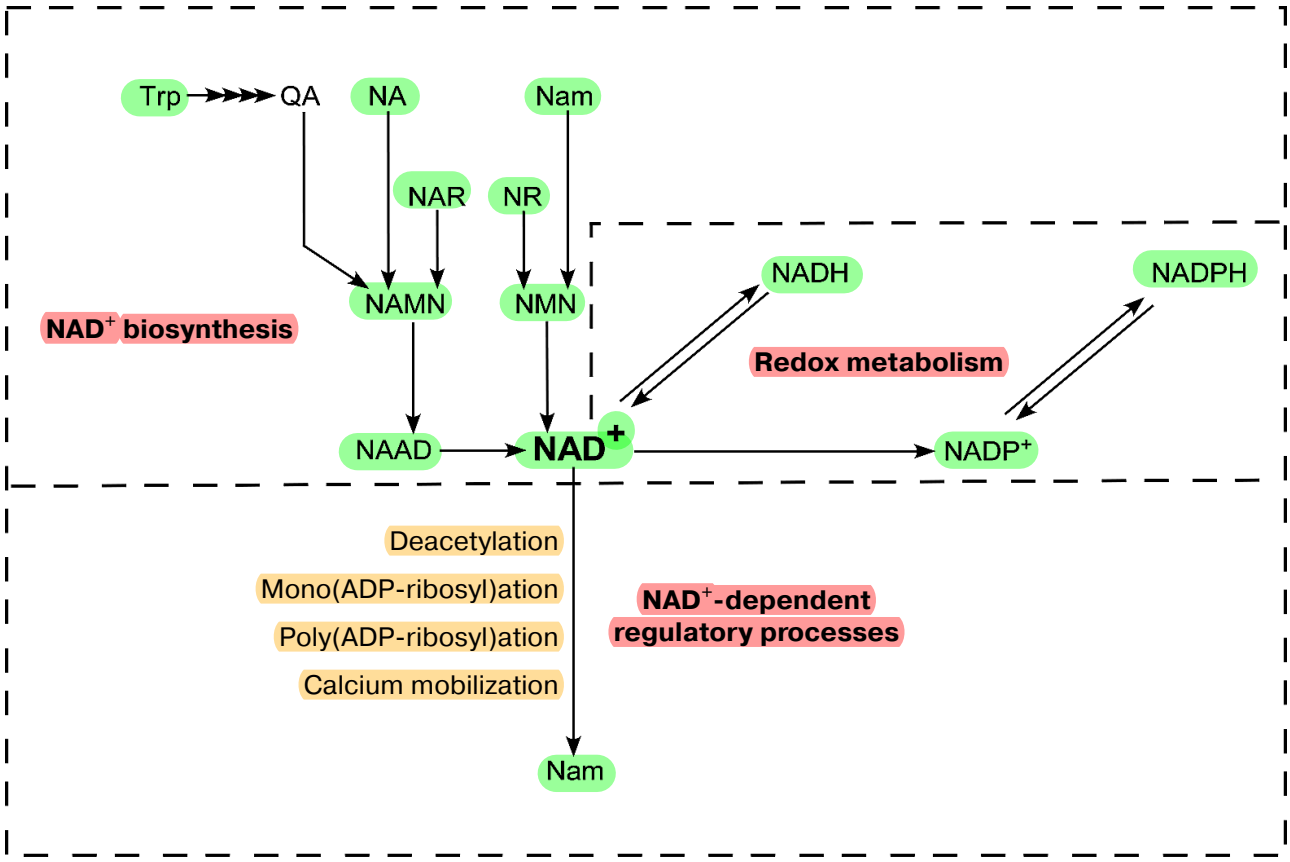

Fig. 1. NAD metabolism in human and animal cells. Quinolinic acid (QA) synthesized from tryptophan (Trp), pyridine bases nicotinamide (Nam) and nicotinic acid (NA), and nucleosides nicotinamide riboside (NR) and nicotinic acid riboside (NAR) are precursors for the synthesis of intracellular NAD^+. First, mononucleotides nicotinamide mononucleotide (NMN) and nicotinic acid mononucleotide (NAMN) are synthesized from all the precursors and then transformed into NAD^+ and nicotinic acid adenine dinucleotide (NAAD), respectively. NAAD is amidated to NAD^+. NAD^+ is phosphorylated to $NADP^+$. In the course of redox reactions, NAD^+ and $NADP^+$ are reversibly transformed into their reduced forms NADH and NADPH, respectively. Deacetylation, mono- and poly(ADP-ribosyl)ation of proteins are NAD^+-dependent modifications. NAD^+ is also used for the synthesis of secondary messengers responsible for cellular calcium mobilization. Nam is cleaved from the dinucleotide as a result of these NAD^+-dependent regulatory processes.

dinucleotide (NAAD), respectively. NAAD is amidated into NAD^+ (Fig. 1) [10].

In this review, we prefer not to discuss the well-known role of NAD as a coenzyme in redox reactions, but rather focus on the less-known regulatory role of NAD^+ in human and animal cells, which has attracted significant attention of researchers in recent years. We also discuss the current status of studies in the field of NAD^+ biosynthesis, compartmentalization of NAD-dependent processes, and modulation of NAD level in cells and tissues in preclinical models of various pathologies.

REGULATORY FUNCTIONS OF NAD

ADP-ribosylation of proteins. ADP-ribosylation is a NAD^+-dependent posttranslational modification of proteins resulting in NAD^+ cleavage into Nam and ADP-ribose with one (mono(ADP-ribosyl)ation) or several (poly(ADP-ribosyl)ation) molecules of ADP-ribose transferred onto specific amino acids of the target protein (Fig. 2a). It has been demonstrated thus far that ADP-ribose can be covalently linked to glutamate, aspartate, lysine, arginine, or serine residues (Fig. 2a) [11, 12]. In the case of poly(ADP-ribosyl)ation, each of the succeeding molecules of ADP-ribose can be attached to the previous one by forming a glycoside bond via hydroxyl groups at carbon atoms of the ribose residue, which results in the formation of either linear or branched polymer consisting of up to 200 ADP-ribose residues (Fig. 2a) [13].

Poly(ADP-ribosyl)ation is catalyzed by the proteins PARP1, PARP2, PARP5a, and PARP5b belonging to the poly(ADP-ribose) polymerase (PARP) family (table) [14]. It is known that poly(ADP-ribosyl)ation plays a crucial role in the cell response to DNA damage. The most extensively studied protein of the PARP family is PARP1, whose activity comprises around 90% of the total PARP

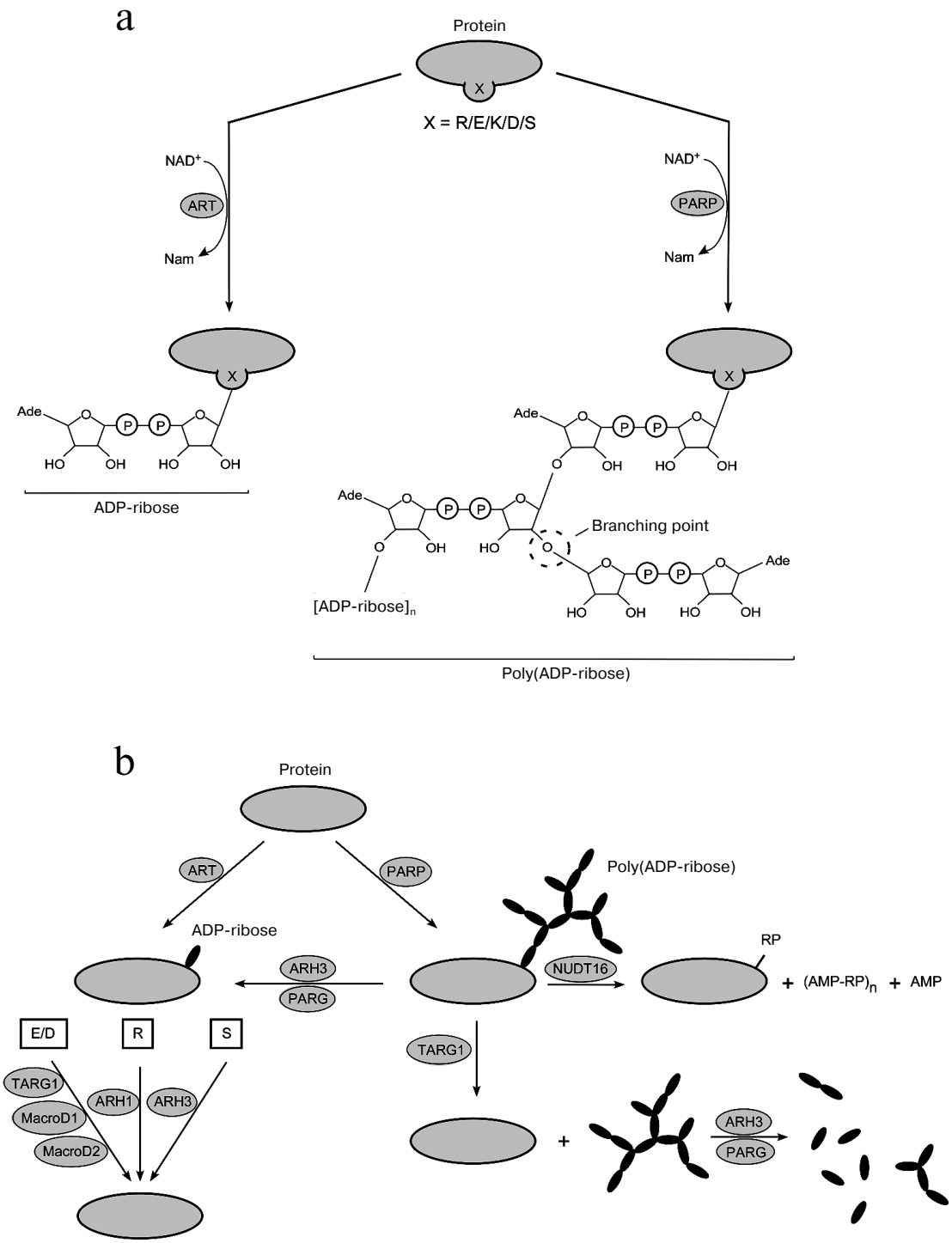

Fig. 2. ADP-ribosylation of proteins. a) Mono- and poly(ADP-ribosyl)ation of proteins. ADP-ribosyltransferases (ARTs) and poly(ADP-ribose) polymerases (PARPs) cleave off Nam from the NAD^+ dinucleotide and transfer the released ADP-ribose either to glutamate (E), aspartate (D), lysine (K), arginine (R), or serine (S) residues of the target protein (mono(ADP-ribosyl)ation) or to the ADP-ribose residue (poly(ADP-ribosyl)ation). Polymers of ADP-ribose can be linear of branched (branching point is indicated). Ade, adenine. b) ADP-ribosylation or proteins and reverse reactions. ARH1 cleaves N-glycosidic bond between ADP-ribose and arginine (R). Glycohydrolase PARG and hydrolase ARH3 cleave O-glycosidic bond in ADP-ribose polymers. Enzymes MacroD1, MacroD2 and glycohydrolase TARG1 remove the remaining molecule of ADP-ribose attached to glutamate (E) or aspartate (D) residues. Hydrolase ARH3 also cleaves N-glycosidic bond between ADP-ribose and serine (S) residue of the modified protein. Nudix hydrolase 16 (NUDT16) cleaves the phosphodiester bond in ADP-ribose or poly(ADP-ribose) attached to the protein. As a result, ribose-phosphate (RP) remains covalently bound to the protein, while AMP and AMP attached to ribose-phosphate (AMP-RP) are released.

NAD^+-dependent protein modifications [1, 32-35]

NAD^+-dependent enzyme	Alternative name	Cell localization	Target protein modification
PARP1	ARTD1	nucleus	poly(ADP-ribosyl)ation
PARP2	ARTD2	nucleus and cytosol	poly(ADP-ribosyl)ation
PARP3	ARTD3	nucleus and cytosol	mono(ADP-ribosyl)ation
PARP4	ARTD4, vPARP	nucleus and cytosol	mono(ADP-ribosyl)ation
PARP5a	ARTD5, TNKS1	cytosol	poly(ADP-ribosyl)ation
PARP5b	ARTD6, TNSK2	cytosol	poly(ADP-ribosyl)ation
PARP6	ARTD17	cytosol	mono(ADP-ribosyl)ation
PARP7	ARTD14, TIPARP	nucleus and cytosol	mono(ADP-ribosyl)ation
PARP8	ARTD16	cytosol	mono(ADP-ribosyl)ation
PARP9	ARTD9, BAL1	nucleus and cytosol	mono(ADP-ribosyl)ation
PARP10	ARTD10	nucleus and cytosol	mono(ADP-ribosyl)ation
PARP11	ARTD11	nucleus and cytosol	mono(ADP-ribosyl)ation
PARP12	ARTD12	cytosol	mono(ADP-ribosyl)ation
PARP13	ARTD13	cytosol	–
PARP14	ARTD8, BAL2	nucleus and cytosol	mono(ADP-ribosyl)ation
PARP15	ARTD7, BAL3	nucleus	mono(ADP-ribosyl)ation
PARP16	ARTD15	cytosol	mono(ADP-ribosyl)ation
ART1	ARTC1	plasma membrane (ectoenzyme), endoplasmic reticulum	mono(ADP-ribosyl)ation
ART2	ARTC2	plasma membrane (ectoenzyme)	mono(ADP-ribosyl)ation
ART3	ARTC3	plasma membrane (ectoenzyme)	mono(ADP-ribosyl)ation
ART4	ARTC4	extracellular	mono(ADP-ribosyl)ation
SIRT1		nucleus and cytosol	deacetylation
SIRT2		cytosol	deacetylation
SIRT3		mitochondria	deacetylation
SIRT4		mitochondria	mono(ADP-ribosyl)ation
SIRT5		mitochondria	deacetylation, demalonylation, desuccinylation
SIRT6		nucleus	deacetylation, mono(ADP-ribosyl)ation, deacylation
SIRT7		nucleus	deacetylation

activity stimulated in response to DNA damage [15]. PARP1 acts as a molecular sensor of single- and double-strand DNA breaks and plays the key role in the repair of these damages [16]. PARP1 binds to the damaged DNA, becomes activated, and poly(ADP-ribosyl)ates itself, histones, and nonhistone chromatin proteins [17, 18]. The negative charge of poly(ADP-ribose) can change the structure of modified proteins and affect their ability to

interact with DNA and other proteins. For example, ADP-ribosylation of histones H1 and H2B results in chromatin relaxation in the region of DNA damage [19, 20], which allows DNA repair complexes to reach the site of damage. Moreover, DNA repair proteins that have poly(ADP-ribose)-binding domains can be recruited directly to the damaged DNA [21].

In the case of extensive DNA damage, large amounts of ADP-ribose polymers are synthesized and migrate from the nucleus to the cytosol, where they stimulate the release of apoptosis-inducing factor (AIF) from the mitochondria intermembrane space. AIF is transported to the nucleus and induces chromosome condensation and activation of DNA-fragmenting endonucleases, thereby initiating caspase-independent programmed cell death [22]. In addition, AIF release from the mitochondria stimulates mitochondrial membrane depolarization and increases permeability of the mitochondrial outer membrane. This results in the cytochrome *c* release from the intermembrane space into the cytosol and initiates caspase-dependent apoptosis [23]. Passive cell death caused by the energy depletion and metabolic exhaustion due to the hyperactivation of PARP1 in response to the massive DNA damage has been also described. It was demonstrated that hyperactivation of PARP1 could decrease the intracellular NAD pool by 90% [24]. As a result of dinucleotide pool depletion, NAD-dependent metabolic processes, such as glycolysis, Krebs cycle, and oxidative phosphorylation, cease to proceed efficiently, which suppresses ATP synthesis and results in the energy depletion and cell necrosis [25].

In addition to its involvement in the cell response to DNA damage, PARP1 plays an essential role in transcription regulation. It was established that PARP1 localizes to the promoters of actively expressed genes and limits H1 histone binding to DNA, thereby maintaining open chromatin structure required for DNA transcription [26]. Furthermore, PARP1 was found to affect transcription activity via poly(ADP-ribosyl)ation of various transcription factors. It was shown that PARP1 poly(ADP-ribosyl)ates and inhibits negative elongation factor (NELF), a protein complex that negatively regulates transcription elongation by RNA-polymerase II, which results in transcription activation [27].

The majority of PARPs are ADP-ribosyltransferases catalyzing mono(ADP-ribosyl)ation of substrates (table). Intracellular mono(ADP-ribosyl)ation of proteins is one of the most important regulatory modifications. For example, cell response to stress caused by accumulation of unfolded proteins in the endoplasmic reticulum (ER stress) involves mono(ADP-ribosyl)ation and activation of IRE1α and PERK kinases by PARP16, resulting in the induction of expression of chaperon genes and translation suppression [28]. It has been found that mono(ADP-ribosyl)ation of the GSK3β kinase by PARP10 suppresses its activity [29]. Mono(ADP-ribosyl)ation is also involved in the transcription regulation. PARP14 protein is associated with the promoters of genes, whose transcription depends of the STAT6 activator. In the absence of interleukin 4, PARP14 recruits HDAC2 and HDAC3 histone deacetylases to promoters. Both these deacetylases inhibit transcription. Under the action of interleukin 4, PARP14 ADP-ribosylates itself and the deacetylases. As a result, HDAC2 and HDAC3 dissociate from the promoters, which facilitates STAT6 binding to the promoters and activates transcription [30].

Intracellular mono- and poly(ADP-ribosyl)ation is also involved in other cellular processes, such as cell cycle control, telomere maintenance, RNA processing, and cell differentiation [2, 4, 5]. In another classification, PARP proteins are assigned to the family of diphtheria toxin-like ADP-ribosyltransferases (ARTDs) (table) [31].

The family of ADP-ribosyltransferases (ART1-4) has also been described that includes enzymes that mono(ADP-ribosyl)ate secreted and membrane proteins, including receptors). Hence, these enzymes can regulate innate immune system responses and cell–cell interactions [36]. According to another classification, these proteins are assigned to the family of clostridial toxin-like ADP-ribosyltransferases (ARTCs) (table) [31].

Mono- and poly(ADP-ribosyl)ation of proteins is a reversible reaction (Fig. 2b). Poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosylhydrolase 3 (ARH3) cleave O-glycosidic bonds in the ADP-ribose polymers [37-39]. Enzymes MacroD1, MacroD2 and terminal ADP-ribose protein glycohydrolase 1 (TARG1) remove the remaining molecule of ADP-ribose attached to the glutamate or aspartate residues (Fig. 2b) [40, 41]. ADP-ribose molecules attached to serine or arginine residues of the acceptor protein are cleaved off by ARH3 or ADP-ribosylhydrolase 1 (ARH1), respectively [42, 43]. Nudix hydrolase 16 (NUDT16) cleaves the phosphodiester bond in ADP-ribose or poly(ADP-ribose) attached to the protein. As a result, ribose-phosphate (RP) remains covalently bound to the protein, while AMP and AMP attached to ribose phosphate (AMP-RP) are released (Fig. 2b) [44].

There has been growing body of evidence that DNA is a common target for ADP-ribosylation as well. PAPR1 and PARP2 catalyze reversible poly(ADP-ribosyl)ation, and PARP3 catalyzes mono(ADP-ribosyl)ation of the double-stranded DNA termini *in vitro* [45, 46]. The biological role of this new type of DNA modification has not been determined yet.

NAD^+-dependent protein deacetylation. Acetylation is one of the key regulatory posttranslational protein modifications. For example, histone acetylation neutralizes positive charge of these proteins and prevents their interaction with DNA, thereby facilitating chromatin decondensation and transcription [47]. Acetylation is catalyzed by the enzymes from the lysine acetyltransferase (KAT) family that transfer acetyl group from

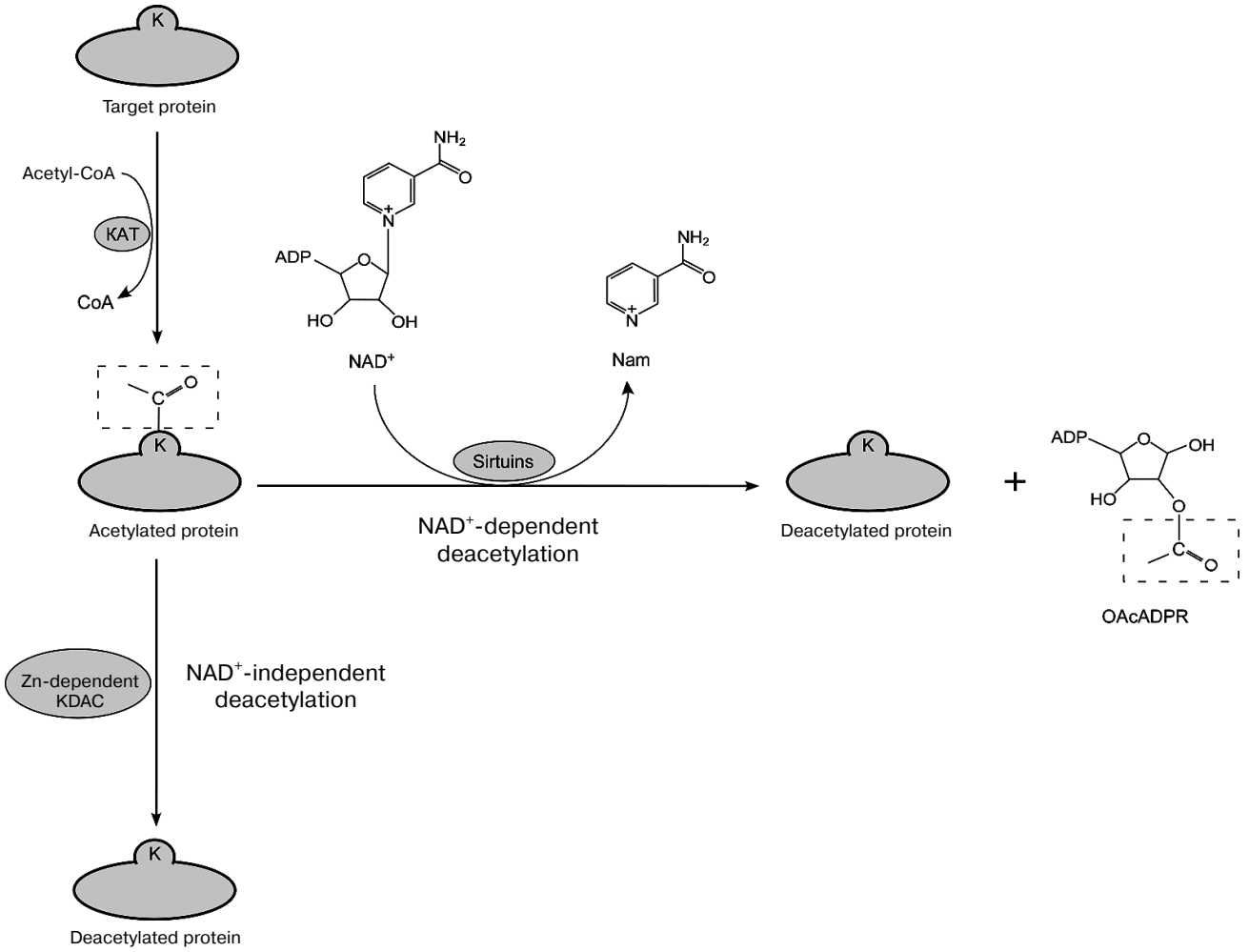

Fig. 3. Acetylation and deacetylation at lysine residues. Lysine acetyltransferase (KAT) catalyzes the reaction of acetyl group transfer from acetyl-CoA to lysine residue (K) of the target protein. During NAD^+-independent deacetylation, Zn-dependent lysine deacetylases (KDACs) remove acetyl group from lysine. In the case of NAD^+-dependent deacetylation, sirtuins cleave Nam from NAD^+ and transfer acetyl group from the lysine residue to the released ADP-ribose, resulting in the formation of O-acetyl-ADP-ribose (OAcADPR).

acetyl-CoA to the lysine residues of the target protein (Fig. 3) [48]. This modification is reversible. Removal of the acetyl groups is catalyzed by lysine deacetylases (KDACs). KDACs are classified into four classes: Zn-dependent deacetylases (classes I, II, and IV) and NAD^+-dependent deacetylases (class III) (Fig. 3) [49].

NAD^+-dependent KDACs belong to the family of highly conserved sirtuin proteins (silent information regulator 2 (Sir2)-like proteins) that have been found in all living organisms from bacteria to humans [50]. These enzymes cleave Nam from NAD^+ and transfer acetyl group from the lysine residue of the modified protein to ADP-ribose with the generation of O-acetyl-ADP-ribose (OAcADPR) (Fig. 3) [51]. At present, seven members of the sirtuin family (SIRT1-7) are characterized in human cells. Sirtuins differ in their intracellular localization and substrate specificity [1]: SIRT1, 6, and 7 have been localized to the nucleus, SIRT2 – to the cytosol, and SIRT3, 4, and 5 – to the mitochondria [52].

NAD^+-dependent KDAC was first identified in yeast cells. Sir2 was initially reported as a component of heterochromatin responsible for the increase in the life-span and suppression of gene expression at the transcription level. Later, the mechanism of transcription regulation by Sir2 was established. It was shown that Sir2 catalyzes NAD^+-dependent deacetylation of histones H3 and H4 resulting in chromatin condensation and suppression of DNA transcription [53].

The closest homolog of the yeast Sir2 in human cells is SIRT1, a NAD^+-dependent histone deacetylase involved in transcription regulation [54]. SIRT1 interacts with transcription repressors and gene promoters. When recruited to the promoters, SIRT1 suppresses gene transcription via deacetylation of histones H3 and H4 [55].

SIRT1 also interacts with histone H1 and deacetylates it, thereby promoting formation of facultative heterochromatin [56].

It has been established that in addition to histones, sirtuins deacetylate a wide array of protein substrates, including regulatory, structural, and catalytically active proteins [57]. NAD^+-dependent protein deacetylation modulates protein activity, stability, localization, and ability to interact with DNA or other proteins.

SIRT1 and SIRT6 play an important role in the cell response to DNA damage. They interact with damaged DNA and participate in the binding and activation of DNA repair proteins. For example, lysine residues of Ku70 protein are acetylated in response to DNA damage, which suppresses the interaction of this protein with Bax and initiates apoptosis [58]. SIRT1 deacetylates Ku70, thus inhibiting apoptosis and stimulating Ku70-dependent DNA repair [59]. SIRT6 deacetylates the CtIP repair factor in response to DNA damage and increases its activity [60].

Hereby, sirtuins participate in the transcription regulation, DNA repair, apoptosis, and maintenance of genome stability. It is also important to note the critical role of sirtuins in the regulation of cell metabolism. The activity of sirtuins depends on the cell energy status, which is defined by various factors including NAD concentration and NAD^+/NADH ratio [61, 62]. Sirtuins participate in the regulation of cell response to external signals via directly connecting the metabolic status of a cell to its signaling mechanisms. For example, SIRT1 deacetylates and activates the key regulator of mitochondria metabolism PGC-1α [63]. Moreover, some essential regulatory proteins, such as FOXO1, FOXO3, NF-κB, PARP1, p53, protein kinase AMPK, and many others, are targets of SIRT1 [1]. Cytosolic SIRT2 deacetylates and activates phosphoenolpyruvate carboxykinase, thereby promoting gluconeogenesis in the case of glucose shortage [64]. SIRT2 also deacetylates α-tubulin [65]. Mitochondrial SIRT3 deacetylates key enzymes of the Krebs cycle, such as glutamate dehydrogenase and isocitrate dehydrogenase, and thus regulates mitochondrial metabolism [66].

In addition to deacetylation, sirtuins also exhibit other catalytic activities (table). Thus, SIRT6 acts as both deacetylase and ADP-ribosyltransferase [67]. SIRT6 can also remove acyl groups of long-chain fatty acid from lysine residues [32]. SIRT5 catalyzes NAD^+-dependent demalonylation and desuccinylation of substrates [68]. SIRT4 modulates activity of its targets (e.g., inhibits glutamate dehydrogenase) through ADP-ribosylation [69].

NAD^+-dependent deacetylases also participate in the regulation of insulin secretion, biological clock regulation, aging, and many other processes [1, 70-72].

Calcium mobilization. NAD^+ and $NADP^+$ are precursors for nicotinic acid adenine dinucleotide phosphate (NAADP), cyclic ADP-ribose (cADPR), and ADPR, which are responsible for cellular calcium mobilization [6]. NAADP and cADPR stimulate calcium release from the intracellular stores; cADPR activates ryanodine receptors and induces calcium release from the ER; NAADP activates two-pore calcium channels in the endolysosomes [73]. ADPR activates calcium transport from the extracellular space through the TRPM2 cation channels in the plasma membrane [74]. All three messengers (NAADP, cADPR, and ADPR) are synthesized in human cells by two glycohydrolases – ecto-ADP-ribosyl cyclase CD38 and its structural and functional homolog CD157 [75]. Considering that CD38 is a type II transmembrane protein with the catalytic site facing the extracellular space, it is still not fully understood how NAADP and cADPR mediate calcium release from the intracellular stores. Moreover, it remains unclear how the availability of NAD^+ and $NADP^+$ (substrates of these ectoenzymes) in the extracellular space is regulated. It was found that NAD^+ can be released from different types of cells via the membrane protein connexin 43 [76]. cADPR can enter the cell via nucleoside transporters [77] or through the channels formed by CD38 oligomer [78]. It was also established that connexin 43 can perform two functions – NAD^+ export and cADPR import to the cell [79]. CD38 could exist as a type III transmembrane protein, with the catalytic domain facing the cytosol, and therefore, can synthesize cADPR in the cytosol [80].

NAD^+ BIOSYNTHESIS

The major pathway for the regulation of the intracellular NAD level is its biosynthesis form the precusors supplied with the diet – bases Nam and NA (vitamin B3), as well as nucleosides NR and NAR (Fig. 4a).

The first step of NAD^+ biosynthesis is generation of mononucleotides NMN and NAMN from all the above-mentioned precursors (Fig. 4b). Nicotinamide phosphoribosyl transferase (NamPRT) catalyzes the reaction between Nam and phosphoribosyl pyrophosphate with the formation of NMN and pyrophosphate. Nicotinic acid phosphoribosyltransferase (NAPRT) converts NA into NAMN. NR and NAR are phosphorylated by the kinases NRK1 and NRK2 to mononucleotides NMN and NAMN [81]. It has been recently demonstrated that the nucleosides NR and NAR are not only supplied with diet but could be also synthesized in cells from NAD intermediates; the suggested mechanism of NR and NAR formation involves dephosphorylation of the mononucleotides NMN and NAMN by cytosolic 5′-nucleotidases [82].

The second step of NAD^+ biosynthesis is the same for all the known precursors. At this step, dinucleotides are formed from the mononucleotides NMN and NAMN. Enzymes from the nicotinamide mononucleotide adenylyltransferase (NMNAT) family catalyze the reactions between NMN and ATP with the formation

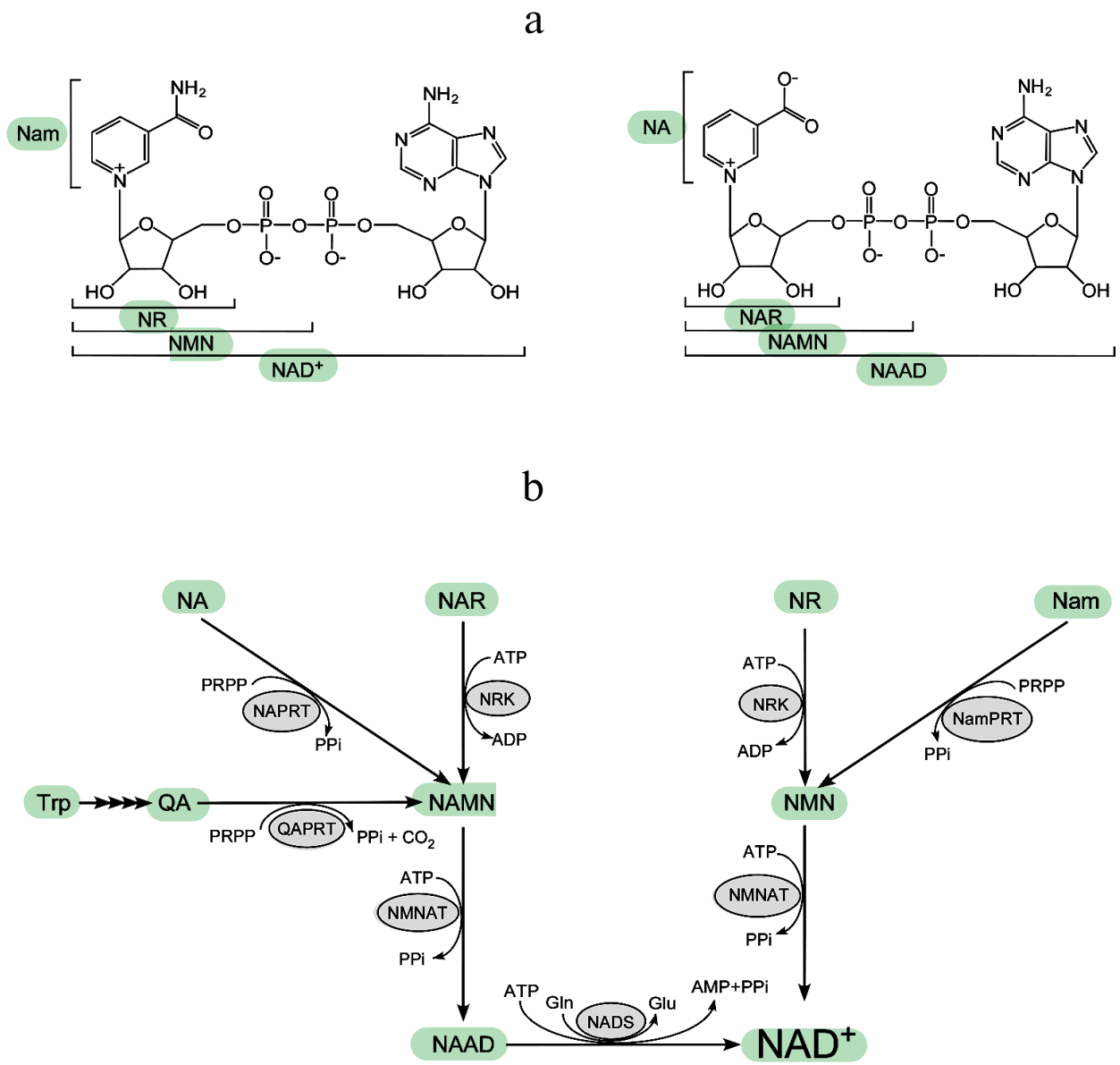

Fig. 4. NAD^+ biosynthesis in human cells. a) NAD^+ and its major intermediates; b) tryptophan (Trp), pyridinic bases Nam and NA, and nucleosides NR and NAR are precursors for the intracellular NAD^+ synthesis. Tryptophan is converted to quinolinic acid (QA) in a series of enzymatic reactions; NAMN is synthesized from QA by quinolinate phosphoribosyltransferase (QAPRT). Nicotinamide phosphoribosyl transferase (NamPRT) converts Nam into NMN, which, in turn, is adenylated to NAD^+ by nicotinamide mononucleotide adenylyltransferase (NMNAT). NA is converted to NAMN by nicotinic acid phosphoribosyltransferase (NAPRT). NAMN is adenylated by NMNAT to NAAD, which is amidated to NAD^+ by NAD synthetase (NADS). Nucleosides NR and NAR are phosphorylated to NMN and NAMN, respectively, by the NRK kinases.

of NAD^+ and between NAMN and ATP with the formation of NAAD (Fig. 4b). The latter is amidated to NAD^+ by NAD synthetase (NADS) that uses glutamine as a donor of NH_2-group (Fig. 4b).

NAD^+ could be also synthesized from tryptophan as a result of several sequential enzymatic reactions. Tryptophan is transformed into quinolinic acid (QA), which is then converted into NAMN by quinolinate phosphoribosyltransferase (QAPRT) followed by the synthesis of NAAD and NAD^+ (Fig. 4b). Since the process involves formation of the pyridine ring, NAD^+ synthesis from tryptophan is considered to be a "*de novo*" pathway [83].

The mechanisms of transport of NAD precursors into human cells are poorly understood. Membrane transporters for tryptophan (SLC7A5 and SLC36A4) and NA (SLC5A8 and SLC22A13) have been identified [84-87], while the import mechanisms for Nam, NR, and NAR remain unknown. Using pharmacological inhibi-

tion, it was found that proteins of the equilibrative nucleoside transporters (ENT) family participate in the NR transport through the plasma membrane [88].

COMPARTMENTALIZATION OF NAD-DEPENDENT PROCESSES

NAD-dependent processes are compartmetalized in a cell. Currently, cytosolic-nuclear and mitochondrial NAD pools have been described in most detailes. Considering that NAD can freely pass through the nuclear pores, the cytosolic and nuclear pools of dinucleotides are combined into one. NAD from the cytosolic-nuclear pool is used in glycolysis, calcium mobilization, ADP-ribosylation, and deacetylation. The mitochondrial NAD pool is required for the oxidation of fatty acids, Krebs cycle, ATP synthesis, and regulation of key mitochondrial enzymes via ADP-ribosylation or deacetylation. There is no exchange between the mitochondrial and cytosolic-nuclear NAD pools in human cells [89, 90]. It is not fully understood how the mitochondrial pool of the dinucleotide is generated. Analysis of intracellular localization of NAD^+ biosynthetic enzymes demonstrated that all of them are localized either to the cytosol or the nucleus with the exception of NMNAT3 – one of the proteins of the NMNAT family [88]. NMNAT3 is located in the mitochondrial matrix; hence, NAD^+ synthesis in mitochondria is likely depends on the NMN transport from the cytosol [88]. However, no mitochndrial transporter for NMN has been found [89]. Using immunochemical detection of poly(ADP-ribose) synthesized as a result of targeted PARP1 expression in different cell compartments, it was demonstrated that in addition to the cytosol-nuclear and mitochondrial NAD pools, there are also dinucleotide pools in peroxisomes, ER, and Golgi apparatus [91, 92]. NAD^+ is transported from the cytosol to peroxisomes by the SLC25A17 transporter [93], where it is used for β-oxidation of fatty acids. In the ER, NADP is required for the first step of the pentose phosphate pathway [94]. The BiP protein that mediates translocation of newly synthesized proteins from ribosomes to the ER and controls their folding in the ER lumen is regulated there via the NAD^+-dependent mono(ADP-ribosyl)ation [95]. The role of NAD in the Golgi apparatus and the mechanism of transport of this dinucleotide to the ER and Golgi apparatus remain unknown.

MODULATION OF NAD LEVELS IN THERAPY

Changes in the NAD levels in cells and tissues are associated with a wide variety of diseases, such as neurodegenerative disorders, diabetes, obesity, metabolic syndrome, cancer, and many others [7-9]. NAD is crucial for vital functions of all cells; hence, modulation of its biosynthesis is a powerful tool in the therapy of different pathologies. For example, reducing the availability of this dinucleotide via inhibition of its synthesis can be used in cancer therapy [8]. Inhibitors (APO866, GMX1777) have been identified and characterized for only one of the known enzymes of NAD^+ biosynthesis – NamPRT catalyzing the first step of NAD^+ synthesis from Nam – and now are tested in clinical trials. Thus, APO866 passed the phase I/II trials for B cell chronic lymphocytic leukemia and phase II trial for melanoma and cutaneous T-cell lymphoma. In the case of GMX1777, the phase I/II clinical trial for metastatic melanoma has been ceased, while the phase I trial for solid tumors or lymphomas was terminated (according to ClinicalTrials.gov from 01.02.2018).

On the other hand, administration of NAD precursors efficiently increases the levels of intracellular NAD, thus preventing the development of various pathologies or improving clinical symptoms during disease progression. Nam and NA (vitamin B3) have been used in humans for a long time as food supplements for normalization of skin conditions, gastrointestinal tract, and nervous system. Nam administration was found to be beneficial for the prophylaxis of glaucoma in aged mice in the mouse model of normal aging [96].

High therapeutic potential of NR has been demonstrated in a series of excellent studies published recently. In particular, it was shown that NR stimulated NAD^+ synthesis in neurons and improved cognitive functions in the mouse model of Alzheimer diseases [97]. Administration of NR prevented neuron degeneration in mouse spiral ganglia and, as a result, protected the mice from the noise-induced hearing loss [98]. Moreover, it was demonstrated for the prediabetes mouse model that NR improved the tolerance to glucose and reduced weight gain, liver cell damage, and hepatic steatosis, as well as prevented development of sensory neuropathy [99]. Similarly, NR significantly decreased glucose levels, reduced weight gain and signs of hepatic stenosis, and prevented the development of diabetic neuropathy in the induced type 2 diabetes mouse model [100]. NR effectively suppressed the development of mitochondrial myopathy at the early and late stages of the disease in mice [101]. NR administration caused marked improvement of the respiratory chain defect and exercise intolerance in a mitochondrial disease mouse model characterized by impaired cytochrome *c* oxidase biogenesis [102]. Currently, NR is used as a food supplement in the human diet (NIAGEN, Chromadex Inc.). The use of NR as a therapeutic agent has been studied actively in recent years: phase I clinical trials have been completed (assessment of NR pharmacokinetics) in healthy individuals and patients with metabolic disorders; phase I/II clinical trials are currently underway in healthy volunteers, patients with prediabetes and obesity, aging patients, and patients with moderate cognitive disorders (according to ClinicalTrials.gov from 02.02.2018).

Administration of NMN produced positive therapeutic effect in the mouse model of induced type 2 diabetes [103], ameliorated the effects of induced ischemia and reperfusion [104], and efficiently mitigated age-associated decline in various physiological parameters (weight, energy metabolism, physical activity, insulin sensitivity, blood plasma lipid profile, vision) [105]. Presently, NMN has a status of food supplement, although the clinical trial of NMN in patients with glucose metabolism disruptions is underway (according to ClinicalTrials.gov from 02.02.2018).

In conclusion, investigation of the mechanisms of action of NAD precursors and development of strategies for their clinical applications are of a high priority in modern biomedicine.

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