

Review

Roles of cADPR and NAADP in pancreatic cells

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Cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) are Ca^{2+} -mobilizing nucleotides that were discovered in the late 1980s. Two decades of investigations have built up a considerable understanding about these two molecules that are related because both are derived from pyridine nucleotides and known to be generated by CD38/ADP-ribosyl cyclases. cADPR has been shown to target the ryanodine receptors in the endoplasmic reticulum whereas NAADP stimulates the two-pore channels in the endo-lysosomes. Accumulating results indicate that cADPR and NAADP are second messenger molecules mediating Ca^{2+} signaling activated by a wide range of agonists. This article reviews what is known about these two molecules, especially regarding their signaling roles in the pancreatic cells.

Keywords cADPR; NAADP; ADP-ribosyl cyclase; Ca^{2+} signaling; Ca^{2+} store; pancreatic acinar cell

Received: March 4, 2012 Accepted: May 4, 2012

Introduction

In the course of evolution, organisms have developed diverse communication systems for maintaining homeostasis, which is essential for life. Signals are delivered by ‘first messengers’, usually hormones and neurotransmitters, which bind to specific receptors that are located in the plasma membrane of target cells and subsequently activate intracellular ‘second messengers’ such as cyclic nucleotides, lipid metabolites, and Ca^{2+} . Ca^{2+} affects nearly every aspect of cellular life including gene expression, proliferation, apoptosis, motility, fertilization, and exocytosis [1]. Decades of intensive investigations have established well-defined concepts of intracellular Ca^{2+} homeostasis and Ca^{2+} signaling. As illustrated in **Fig. 1**, two classes of Ca^{2+} transporting/exchanging systems are present on almost all kinds of cell membranes. One class (marked with red arrows) consists mainly of Ca^{2+} channels, which, in response to stimuli, can increase the cytosolic Ca^{2+} concentration by releasing Ca^{2+} from intracellular stores or by

promoting Ca^{2+} influx from the extracellular medium that normally is high in Ca^{2+} . Another class (marked with blue arrows) consists mainly of Ca^{2+} transporters and ATPases which can reduce cytosolic Ca^{2+} concentration by various energy-dependent pumping or exchange processes. The net effect of all these events is to maintain the cytoplasmic resting intracellular Ca^{2+} concentration at ~ 100 nM, substantially lower than the millimolar concentrations in the exterior medium.

Ca^{2+} dynamics and homeostasis are critically important for the endocrine and exocrine functions of the pancreas, which depend respectively on two different populations of cells, acini and islets of Langerhans. Acini secrete pancreatic juice containing digestive enzymes that assist in the digestion and absorption of nutrients in the small intestine. In contrast, the islets secrete several important hormones, including insulin, amylin, glucagon, somatostatin, and pancreatic polypeptide.

Ca^{2+} plays a key role in controlling secretory processes of both the exocrine and endocrine pancreas. In the exocrine pancreas, secretagogues, such as cholecystokinin (CCK) and acetylcholine (ACh), induce specific and spatiotemporally distinct Ca^{2+} oscillations [1]. In fact, the pancreatic acinar cell is a classic model for the study of exocytosis and Ca^{2+} -signaling pathways. The literature about the role of Ca^{2+} in the endocrine pancreas is less pervasive but focuses particularly on the important role of Ca^{2+} in insulin secretion [2]. Recent and intensive studies have revealed the surprising roles of two novel Ca^{2+} -mobilizing messengers, cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), in the pancreatic system. This review will summarize the advances in this field.

Discovery of cADPR and NAADP

In 1983, Streb *et al.* [3] discovered the Ca^{2+} -mobilizing activity of inositol 1,4,5-triphosphate (IP_3), which was shown subsequently to control a multitude of cellular responses [4]. Although IP_3 production was critical for many cellular responses, it did not account for all aspects of intracellular

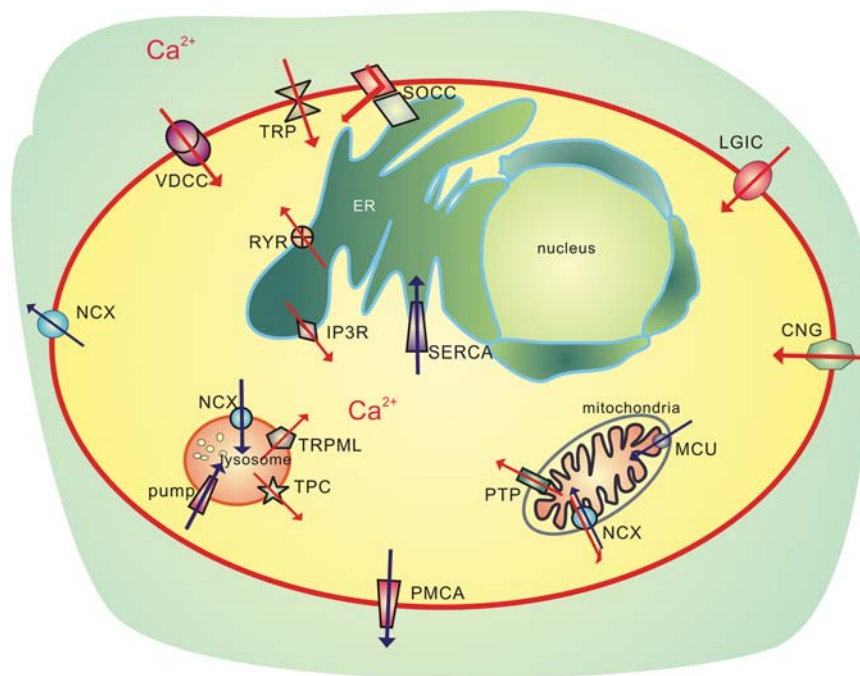


Figure 1 Elements involved in Ca^{2+} dynamics and homeostasis Plasma membrane: voltage-dependent calcium channels (VDCCs); transient receptor potential channel (TRPs); store-operated Ca^{2+} channels (SOCCs); cyclic nucleotide-gated ion (CNG) channels; ligand gated ion channels (LGIC) such as P2X receptors; $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX); plasma membrane calcium-transporting ATPase (PMCA), etc. ER: IP3 receptors (IP3Rs); ryanodine receptors (RYRs); sarco/endoplasmic reticulum Ca^{2+} -ATPases (SERCA), etc. Mitochondria: mitochondrial calcium uniporter (MCU); permeability transition pore (PTP); $\text{Na}^+/\text{Ca}^{2+}$ exchanger (mNCX), etc. Endo-lysosome: two-pore channels (TPCs); mucolipin family of transient receptor potential channels (TRPMLs); NCX; pumps (PMCA-like and SERCA 3) etc.

Ca^{2+} release. Particularly puzzling was how cells are able to distinguish different stimuli and generate diverse spatio-temporal calcium signals if they possess only a single Ca^{2+} messenger, IP_3 . This made investigators search for other possible Ca^{2+} -mobilizing second messengers. One system that proved fruitful in the search for calcium messengers is the sea urchin egg. The egg exhibits one of the most spectacular Ca^{2+} waves during fertilization, which is due almost exclusively to release from internal Ca^{2+} stores [5,6]. The stores are abundant and can be easily isolated from cell homogenates and developed into an *in vitro* bioassay for Ca^{2+} -releasing messengers [6]. It had been shown previously in the egg that early metabolic events associated with fertilization included changes in pyridine nucleotides, with the pool of NAD prominently decreased while that of NADP increased [7]. This had prompted the testing of the possible roles of both NAD and NADP in mediating Ca^{2+} release in the egg. Indeed, it was found that both can activate Ca^{2+} release from stores in the homogenates, with the effect of NAD showing a clear time delay, whereas the release by NADP or IP_3 was much more immediate (**Fig. 2**) [6].

As shown in **Fig. 2**, addition of NAD to sea urchin egg homogenate induced a delayed calcium release, which turned out to be due to the enzymatic conversion of the inactive precursor, NAD, to the active metabolite, cADPR.

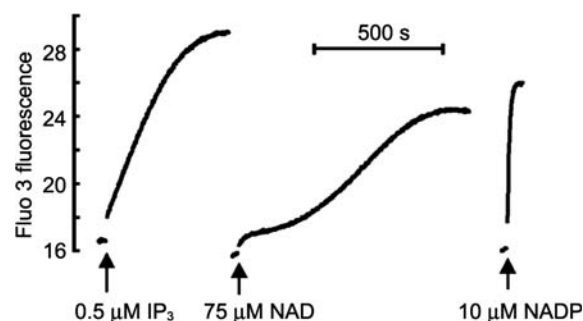


Figure 2 Discovery of cADPR and NAADP The ability of NAD(P) to release Ca^{2+} was first demonstrated using homogenates isolated from sea urchin eggs. The release of Ca^{2+} was detected by the Ca^{2+} indicator, Fluo-3, as increase in fluorescence. The amounts of Ca^{2+} release were comparable with that released by IP_3 , a known Ca^{2+} -mobilizing messenger. The initial delay in Ca^{2+} release induced by NAD was shown to be due to its enzymatic conversion to cADPR, while the response to NADP was due to a contaminant in NADP that was later identified as NAADP.

Using various radioactive NAD precursors as substrates for the reaction, it was shown that the active molecule retained essentially all parts of NAD, including the adenine ring, the phosphoryls, and the ribose residues, but not the nicotinamide group, and yet it was different from all known metabolites of NAD, such as ADP-ribose or AMP. The structure of cADPR was ultimately determined by X-ray crystallography and shown to be a novel cyclic molecule

with the adenine ring of NAD linked back to the terminal ribose forming a complete circle [8,9].

Equally novel but even more surprising, the Ca^{2+} -mobilizing activity of NADP was shown to be actually due to the contamination of commercial preparations by another compound, NAADP (Fig. 2). Except for the small structural change of the amide group replaced by a carboxyl, NAADP is identical to NADP, and yet it was the most potent compound found to release Ca^{2+} , with a half-maximal effective concentration around 30 nM [10].

cADPR and NAADP as Second Messengers in the Pancreas

After the discovery that cADPR and NAADP can mobilize Ca^{2+} in sea urchin eggs [6,8,10], these compounds were subsequently shown to have similar activity in diverse cells including rat pituitary [11], rat dorsal root ganglion neurons [12], protists [13], and plants [14] (reviewed in [15]).

The first demonstration of a role of cADPR in the endocrine pancreas was published by Okamoto's lab in 1993 [16]. Using digitonin-permeabilized mouse islets as the cell system, they showed that cADPR played a key role in stimulus-secretion coupling. In a series of subsequent studies, they further showed that (i) transgenic mice overexpressing human CD38, a cADPR-synthesizing enzyme (detailed below), produced more cADPR, and had increased glucose- and ketoisocaproate-induced insulin secretions [17]; (ii) CaMKII was required for the activation of the ryanodine receptor (RyR), the known target of cADPR [18]; and (iii) cADPR-induced regulation of the RyR was indirect, via binding to its accessory protein, FK506-binding protein 12.6 (FKBP12.6) [19].

However, negative results from other groups suggested that cADPR did not play a role in glucose-induced insulin secretion [20–25]. It is now accepted that some of the discrepancies may have resulted from differences in the species and methodologies used. Results from several independent groups have confirmed the role of cADPR in insulin secretion [26,27]. Further clarification of the issue is likely with more experiments.

In contrast, the situation in the exocrine acinar cells has been much more straightforward. A positive role of cADPR in the acinar cells was reported by many groups [28–36]. Intracellular application of cADPR induced repetitive Ca^{2+} spikes localizing mainly in the secretory pole, and these spikes were blocked by antagonists of both RyR (ryanodine) and IP₃R (heparin) [36]. In streptolysin-O permeabilized rat acinar cells, the cADPR-induced Ca^{2+} signals were specifically abolished by a cADPR antagonist, 8-NH₂-cADPR, which also blocked the CCK-induced Ca^{2+} spikes, indicating that the CCK signaling is mediated by cADPR [25]. Consistently, the intracellular level of

cADPR was found to be elevated by CCK treatment [32]. Similar to CCK, ACh, another secretagogue, could also induce Ca^{2+} spikes that are mediated by cADPR. In mice with the cADPR-synthesizing enzyme, CD38, ablated the ACh-induced cADPR production was abolished [33], further establishing the second messenger role of cADPR in pancreatic acinar cells.

The Ca^{2+} signaling in acinar cells was shown, in fact, to be mediated by multiple Ca^{2+} messengers. Not only cADPR and IP₃, but NAADP is also involved. The fact that NAADP can stimulate Ca^{2+} release in acinar cells was first reported by Galione's group in 1999 [37]. They demonstrated that a low concentration (50 nM) of NAADP triggered Ca^{2+} spikes, whereas higher concentrations (1–100 μM) of NAADP did not. These results were consistent with previous reports of a self-desensitization property of NAADP in the sea urchin egg [38,39]. Unexpectedly, high concentrations of NAADP that caused self-desensitization were found to selectively inactivate CCK-evoked Ca^{2+} signals as well [37], suggesting that NAADP, in addition to cADPR, was also involved in the CCK signaling. Consistently, CCK stimulated the elevation of not only cADPR but also NAADP. The increase in NAADP, in fact, preceded that of cADPR [32]. To account for these findings, it was proposed that NAADP may function as a Ca^{2+} trigger to initiate the CCK signaling, whose signal is then amplified subsequently by the cADPR and IP₃ pathways [37,40].

Evidence showed that NAADP also played a key role in the endocrine pancreas. Photolysis of injected caged NAADP to elevate intracellular NAADP in β cells activated Ca^{2+} increases similar to those stimulated by glucose, whereas high concentrations of NAADP blocked the glucose-induced Ca^{2+} changes [41,42]. Consistent with these results, the newly developed antagonist of NAADP, Ned-19, blocked both the NAADP- and glucose-induced Ca^{2+} increases in mouse pancreatic β cells [43]. As described below, NAADP mobilizes Ca^{2+} from the acidic Ca^{2+} stores in cells. Inhibitors of the Ca^{2+} transport in acidic stores likewise blocked both the NAADP- and glucose-induced Ca^{2+} changes [41]. Direct measurements showed that glucose could indeed induce elevation of endogenous NAADP in the islets [27].

Robison *et al.* [44] established the criteria that signaling molecules, such as cADPR and NAADP, must satisfy before they can be considered as second messengers. Consistent with these criteria, it has been shown that (i) intracellularly applied cADPR/NAADP mimic the effect of the extracellular stimulus, such as CCK or ACh; (ii) enzymes, such as CD38, which synthesize and metabolize cADPR/NAADP in mammalian cells have been identified and characterized; (iii) intracellular levels of cADPR and NAADP are shown to change in response to the

extracellular stimuli; and (iv) specific antagonists of cADPR and NAADP, such as 8-NH₂-cADPR or Ned-19, can block the effects of the stimuli. With all these criteria satisfied, it is thus fair to conclude that cADPR and NAADP are second messengers for Ca²⁺ signaling.

Targets of cADPR

Studies in sea urchin eggs indicated that the Ca²⁺-releasing mechanism of cADPR was different from that of IP₃ but similar to the Ca²⁺-induced Ca²⁺ release mediated by the RYR on the endoplasmic reticulum (ER) membrane [45]. Further experiments by different groups, using reconstituted RYR in lipid bilayers [46], in myocytes that express RYR [47], or in RYR overexpressed HEK-293 cells [48], showed that cADPR released Ca²⁺ from the ER pool via RYR. The action of cADPR on RYR, however, was found to require some accessory proteins such as calmodulin (CaM) [49–51] and FKBP12.6 [19,52–55]. The requirement for accessory proteins could account for some of the controversy about the action of cADPR (reviewed in [56]), as uncontrolled loss of these soluble factors could lead to the negative results reported in the literature. Besides ER, cADPR has also been shown to target other Ca²⁺-handling machineries, such as the sarcolemma Ca²⁺ pump (SERCA), to promote Ca²⁺ removal in smooth muscle [57], and the Ca²⁺-influx channel, TRPM2 [58,59].

Consistent with cADPR having a key role in the pancreas, the dominant targets of cADPR, the RYRs, were shown to be expressed in both endocrine and exocrine pancreas [25,60]. In the islets, Okamoto's group showed that the activating effect of cADPR on RYR required FKBP12.6, and was mediated via a CaM-dependent kinase [18,19]. In the acini, pharmacological studies, likewise, showed that cADPR indeed targeted the RYRs [61] and its action also required CaM [62]. In rat insulinoma RIN-5F cells and islets, cADPR was shown to activate the TRPM2 channel in a temperature-dependent manner [59].

Targets of NAADP

The Ca²⁺-mobilizing properties of NAADP were shown to be totally different from those of cADPR and IP₃, as the effects of NAADP were insensitive to all known inhibitors of IP₃R, RYR, and SERCA [6,10,63]. The action of NAADP showed an unusual property of self-desensitization at high concentrations, which has been used as a diagnostic tool to authenticate the effect of NAADP [38,39]. Subsequently, a specific antagonist, Ned-19, was developed for a similar purpose [43]. Early studies in sea urchin egg showed that NAADP targeted acidic organelles [64,65]. More recent work confirmed that the lysosomes in mammalian cells are the most likely targets of NAADP [42,66].

Isolated lysosomes have been used to demonstrate the direct action of NAADP by using a glass chip-based technique [67]. Other possible targets of NAADP have also been reported in a few studies including mucolipin 1 [68] and RYR [69,70].

Recently, the two-pore channels (TPCs), the poorly characterized members of the voltage-gated cation channels family, have been proposed to be the receptors for NAADP. Their lysosomal localization first attracted attention and they turned out to be the most convincing candidates, as they meet many of the properties of the NAADP-gated Ca²⁺ mechanism [71–74]. Three members in this family were identified and TPC2, localized on lysosomes, was the first shown to be responsive to NAADP [72]. NAADP binding also increased in cells overexpressing TPC2. Ablation of TPC2 or addition of inhibitors of lysosomal Ca²⁺ transport abolished the NAADP-dependent mobilization of the lysosomal Ca²⁺ stores [72].

TPC1 is present throughout the endocytic pathway, from endosomes, late endosomes to lysosomes. NAADP-mediated calcium signals were enhanced after overexpression of TPC1 and attenuated after knockdown of TPC1, and mutation of a single highly conserved residue within a putative pore region of TPC1 abrogated calcium release by NAADP [73]. Thus, TPC1 was critical for NAADP action as well.

TPC3, on the other hand, is expressed throughout the deuterostome lineage but is a pseudogene in humans and other primates, and when overexpressed in mammalian cells, it also localizes to acidic organelles [74,75]. However, the response of TPC3 to NAADP is controversial. One group found overexpression of TPC3 from the purple sea urchin (*Strongylocentrotus purpuratus*) in mammalian cells mediated NAADP-dependent calcium release [74], whereas another group claimed that the expression of TPC3, from the same species, suppressed NAADP-induced Ca²⁺ release [75]. Further investigations are needed to settle this controversy.

In the isolated pancreatic β cells of TPC2^{-/-} mice, NAADP failed to activate the cation currents, strongly suggesting that native NAADP-evoked Ca²⁺ signaling is mediated by TPC2 in β cells [72]. Although the role of TPCs in acinar cells has not been reported yet, the fact that NAADP is known to be important suggests that loss of expression of TPCs will likely lead to functional impairment of the exocrine pancreas as well.

Biogenesis of cADPR and NAADP

cADPR was first identified as a metabolite of NAD that was formed by an unknown enzyme from extracts of sea urchin egg and animal tissues [76]. Later, an enzyme was purified from *Aplysia californica* and shown to catalyze the conversion of NAD to cADPR with the release of the

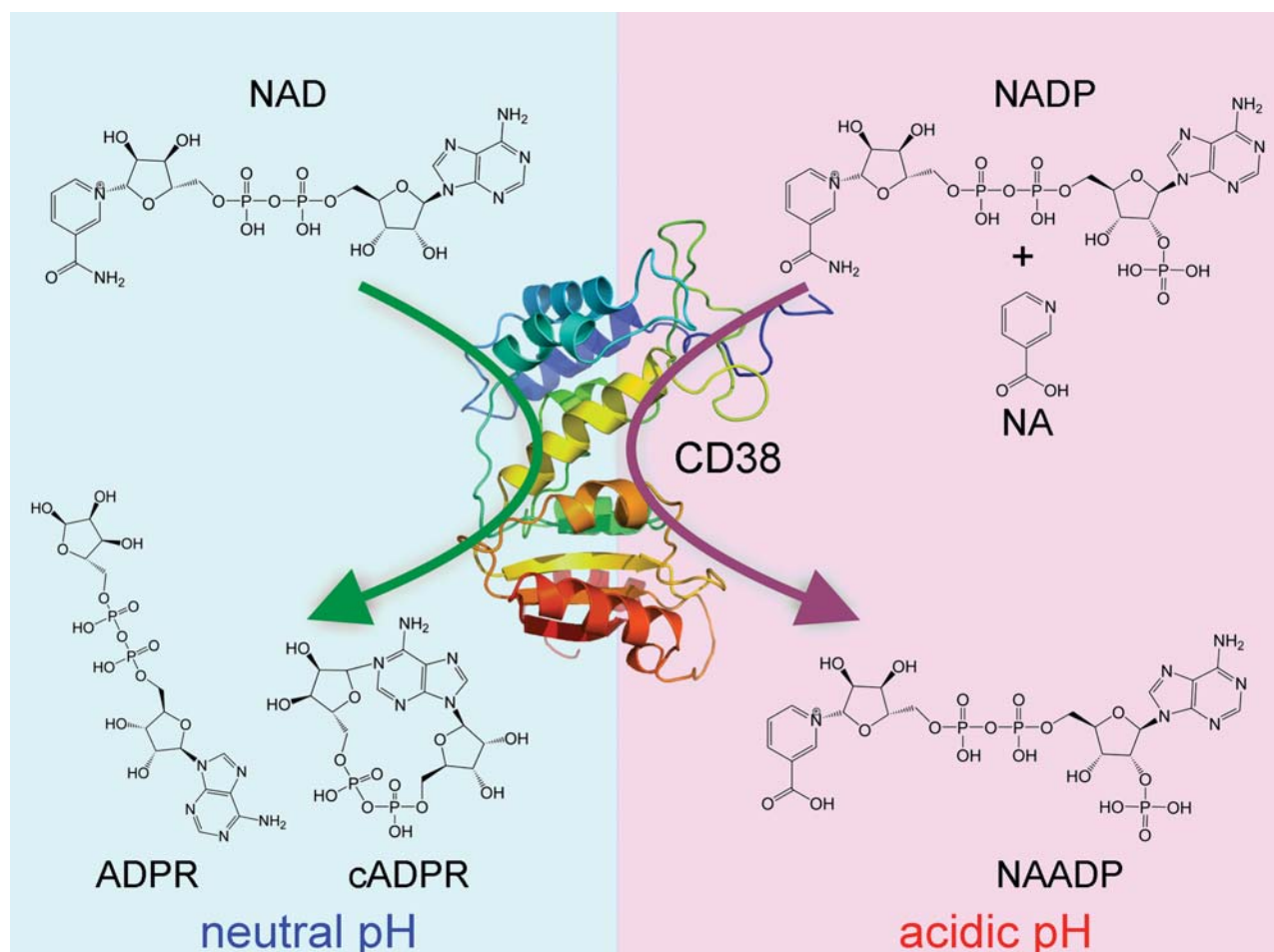


Figure 3 The major enzymatic reactions catalyzed by CD38 The chemical structures of the substrates and products of the reactions are shown.

nicotinamide group. The novel enzyme was named as ADP-ribosyl cyclase [77]. A homologue of ADP-ribosyl cyclase in mammalian cells was identified as CD38 [78]. Further studies revealed that CD38 is a multifunctional enzyme (**Fig. 3**), which not only catalyzes the cyclization of NAD to produce cADPR, but also hydrolyzes cADPR to produce ADPR [79], as well as catalyzing a base-exchange reaction at acidic pH to produce NAADP in the presence of nicotinic acid [80].

The catalytic mechanism for CD38 has been actively studied [81,82]. It was first identified as a cell surface differentiation marker in B lymphocytes [83] and later found to be expressed ubiquitously. It is a type-II membrane protein ($N_{in}-C_{out}$) with the catalytic C-terminal domain having an extracellular localization. The localization of the enzyme activity to the extracellular space presents a topological problem because the substrates, products, and the Ca^{2+} stores targeted by cADPR and NAADP are located in the cytosol.

Active research is currently conducted to resolve this 'topology paradox' [84]. De Flora's group was the first to propose a transporter model [**Fig. 4(A)**], in which NAD was first transported out by connexin 43 (Cx43) [85] and,

after its conversion by CD38, cADPR, the product, was transported back into the cell by nucleoside transporters present in the plasma membrane [86]. This model seems awkward but has been supported by several independent studies [87–89].

We have proposed another model, called the 'type-III CD38 model' [**Fig. 4(B)**], in which a small amount of type-III ($N_{out}-C_{in}$) CD38 coexists with the type-II ($N_{in}-C_{out}$) CD38 on cell membranes. The type-III CD38, with its catalytic domain inside the cytosol is thus amenable to be controlled by intracellular regulatory systems such as protein kinases, interacting proteins, etc. This possibility is novel but may offer a more straightforward resolution to the conundrum. Indeed, the existence of an extracellular location of the N-terminus of CD38 has been observed in a β -cell line, MIN6. Furthermore, external ligation of the N-terminal located flag-tag of the type-III CD38 by a specific antibody can induce enhanced insulin secretion [90].

CD38 is not only expressed on the cell surface but also in intracellular organelles [91,92]. Nuclear CD38 was also found in a type-III orientation, in which the catalytic C-domain is in the nucleoplasm [93,94]. Further support of

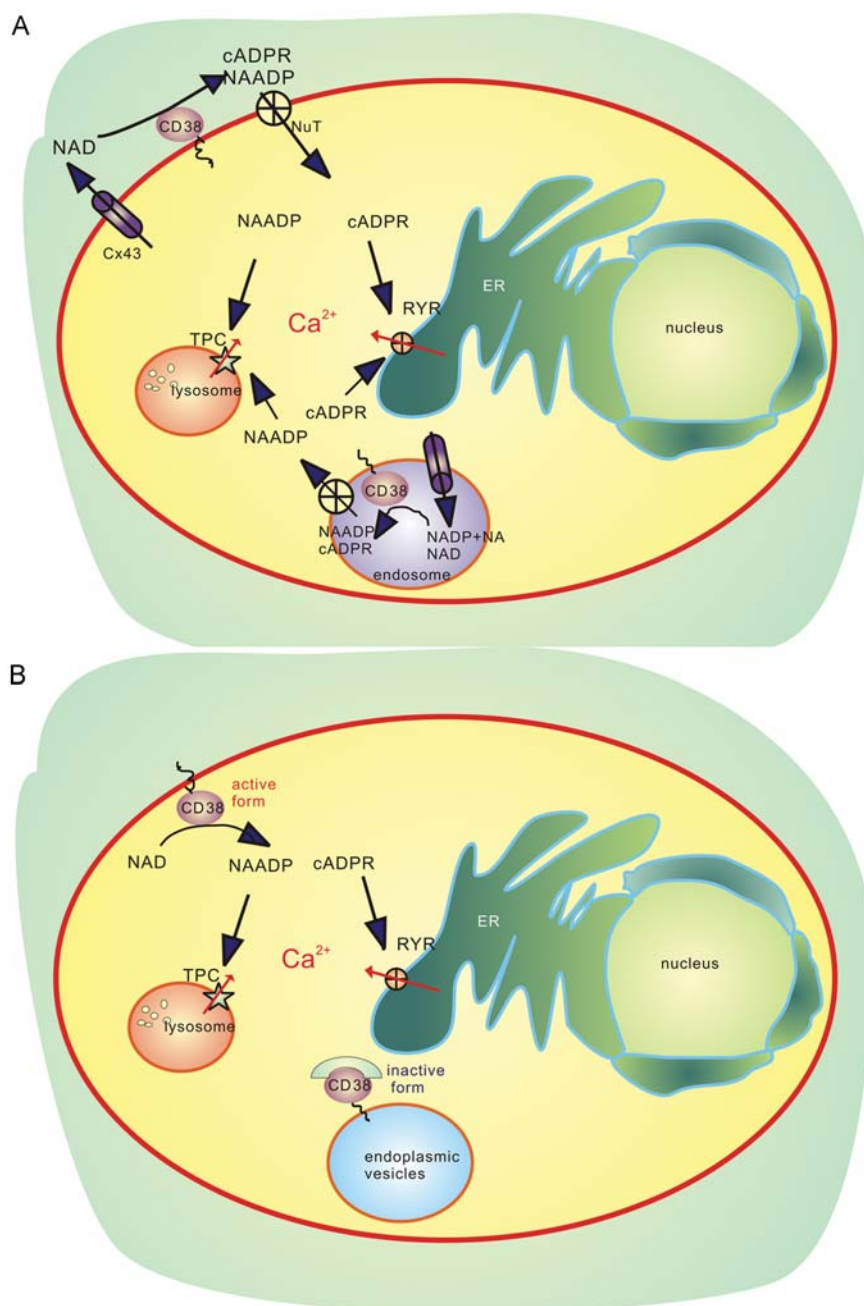


Figure 4 Schematic models for the cADPR/NAADP/CD38-signaling pathway (A) Transporter model. NAD leaks out or enters endosome via Cx43 channels. After synthesis by CD38, the products, cADPR and NAADP, are transported back into the cytosol by nucleoside transporters (NuT) and target the RYR to release Ca^{2+} from ER and TPCs in the endo-lysosomes, respectively. (B) Type-III CD38 model. A small amount of type-III (N_{out}-C_{in}) CD38 coexists with the type-II (N_{in}-C_{out}) CD38 on the cell membrane and the activity is controlled by intracellular regulatory systems such as protein kinases or interacting proteins. NAD reaches CD38 and is catalyzed to produce cADPR/NAADP, which act on their targets and release calcium. All the reactions take place in the same space, the cytoplasm.

the type-III CD38 hypothesis comes from our recent study that shows that the expression of CD38 intracellularly, which is in a reduced environment, does not impede its enzymatic activity, which requires disulfide bonds [95]. The hypothesis that type-II membrane proteins could escape from the ER by a transport process mediated by lipid droplets [96] provides another possibility for post-translational switching of the type-II CD38 to type-III

membrane orientation with access to the substrates in the cytoplasm.

Although the final details of the biogenesis of cADPR and NAADP are still to be elucidated, the involvement of CD38 seems obvious according to studies with CD38^{-/-} mice, in which the endogenous levels of cADPR in both pancreatic β cells and acinar cells and also neutrophils [33,58,97] and the agonist-induced synthesis of NAADP

[98,99] were all found to be greatly reduced. That the base-exchange reaction catalyzed by CD38 to produce NAADP requires acidic pH may seem unnatural. It is so only if it occurs in the cytosol, but is perfectly natural if it occurs inside lysosomes/endosomes as postulated by the transporter model [Fig. 4(A)]. Clearly the possibility that NAADP could be produced by reactions other than the base-exchange reaction cannot be excluded. In fact, CD38-independent production of NAADP has been reported in myometrial cells [100]. The study also proposed that NAADP might be generated by deamidation of NADP or phosphorylation of NAAD by NAD kinase. However, the role of NAD kinase has already been ruled out [101], whereas deamination of NADP has never been successfully demonstrated.

cADPR- and NAADP-mediated Signaling Pathways in the Pancreas

The primary function of pancreatic acinar cells is to synthesize and secrete digestive enzymes that are responsible for digestion of nutrients. Both synthesis and secretion are controlled by the vagal nerve, whose postganglionic neurons release ACh and, in some species, other peptides including the gastrointestinal hormones CCK and bombesin.

CCK, ACh, and bombesin bind to their own specific receptors and act through heterotrimeric G proteins to produce messengers that act to release intracellular Ca^{2+} . Among these messengers, IP_3 is the most extensively studied; following receptor stimulation, it is produced by activation of phospholipase C, and it acts on IP_3 receptors to release Ca^{2+} from the ER stores [4]. As described above, both cADPR and NAADP are also produced and act on the RYRs in the ER and TPCs in the endolysosomal compartment, respectively [32]. The decrease of the Ca^{2+} concentration in the ER lumen could also secondarily activate the store-operated Ca^{2+} channels and induce Ca^{2+} influx [102]. These three messengers thus act like an orchestra, in which different messengers are responsive to different stimuli. Their production and actions are also spatially and temporally distinct. CCK uses the NAADP/TPC pathway to produce local Ca^{2+} changes. This triggering Ca^{2+} signal is then amplified by the $\text{IP}_3/\text{IP}_3\text{R}$ and cADPR/RYR mechanisms to produce global Ca^{2+} changes and generate oscillations. In contrast, bombesin and ACh activate production of cADPR and IP_3 only, but not NAADP, and produce global Ca^{2+} changes and oscillations directly [28,61,103].

The islets of Langerhans form a nutrient-sensing network that spreads throughout the pancreas. The pancreatic β cells, the most abundant islet cell type, can detect elevated blood glucose levels and secrete insulin accordingly. They are also tightly controlled by other hormones, such as glucagon-like peptide-1 (GLP-1).

As no glucose receptor has been found, it is thought that glucose acts via a non-receptor-mediated process [104]. High blood glucose quickly enters the β cells via the GLUT2-facilitated transport and is phosphorylated by glucokinase, which acts as a glucose sensor. A cascade of metabolite elevations is initiated as a result of glucose metabolism, resulting in increased generation of ATP and other nucleotides. A model has been proposed that the generation of ATP is associated with an increase in the ATP/ADP ratio, thereby leading to closure of the K_{ATP} channel and membrane depolarization. This, in turn, activates the voltage-dependent calcium channels, Ca^{2+} influx, cumulating in a rise in cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and the activation of the exocytotic machinery [104]. This model that is based solely on the K_{ATP} channel is incomplete as genetic ablation [105] or blocking [106] of the channel does not eliminate the glucose-induced insulin secretion in the islets.

The fact that three Ca^{2+} messengers are present and fully functional in β cells suggests that mobilization of intracellular Ca^{2+} stores should play a key role in glucose-signaling, in addition to the Ca^{2+} influx via the K_{ATP} mechanism. Among the three second messengers, the function of IP_3 has been poorly documented, while that of cADPR's involvement is controversial [16,23]. The most promising and exciting possibility focuses on the contribution of NAADP/TPC pathway [107]. First, intracellular application of low concentrations of NAADP-evoked oscillations and high concentrations suppressed the glucose-evoked Ca^{2+} oscillations [41,43]. High affinity-binding sites for NAADP were found in the islets and glucose evoked an increase in cellular NAADP levels [41]. Bafilomycin and Ned-19 abolished glucose-induced responses but not those induced by ACh, whereas thapsigargin abolished the ACh-induced response but not the glucose responses [42,43], which also indicates that the ER store might not play an essential role in glucose-stimulated Ca^{2+} signaling. The study of glucose-mediated Ca^{2+} signaling in the β cells of $\text{TPC}^{-/-}$ mice will provide important insight about the role of NAADP in β -cell Ca^{2+} signaling.

High blood glucose induces secretion of insulin from β cells, which acts by a feedback mechanism on β cells via autocrine or paracrine ways. Binding of insulin to its receptor activates a kinase cascade as well as Ca^{2+} changes. NAADP also plays a key role in insulin signaling. Thus, desensitizing the NAADP-mechanism by injecting high concentrations of NAADP abolished the insulin-evoked Ca^{2+} signals [108]. Ned-19 abrogated the insulin-stimulated ERK phosphorylation [109]. Insulin-induced production of NAADP, and also cADPR, was found to be at least partially dependent on CD38, as shown by a CD38-knockout study [110].

GLP-1, an incretin, is secreted in a nutrient-dependent manner and stimulates glucose-dependent insulin secretion, as well as promotes the proliferation of β cells [111]. Similar to insulin, activation of the GLP-1 receptor stimulates kinase cascades and increases $[Ca^{2+}]_i$. Previous studies show that GLP-1 stimulation is coupled to both cADPR and NAADP synthesis, culminating in Ca^{2+} release from ER and acidic stores [27]. These effects of GLP-1 are observed in the presence of high glucose and represent a separate and/or additional response to that of glucose as described above [27]. The initial phase of the GLP-1-activated Ca^{2+} signals is ascribed to NAADP, while the second and the maintained phase is due to the cADPR pathway, but not the IP_3 pathway. In support of these data, β cells isolated from CD38^{-/-} mice showed reduced production of NAADP and cADPR activated by GLP-1, indicating partial dependence on CD38 [27].

Conclusion

In the last two decades, the signaling functions of cADPR and NAADP have been progressively elucidated. The structures and *in vitro* catalytic mechanisms have been well established. Their Ca^{2+} mobilizing activities have been confirmed in a variety of cells and their targets and mechanisms have been shown to be totally different. NAADP activates TPCs to mobilize the endo-lysosomal Ca^{2+} store, while cADPR activates RYRs and releases Ca^{2+} from the ER pool. Sometimes they work together: NAADP as a trigger and cADPR (as well as IP_3) as an amplifier. They can also act independently, with each linked to a specific agonist. They, like artists in an orchestra, play the music of Ca^{2+} according to the signals from the conductor, stimulus. The pancreas provides a stage for these players, where exocytosis of enzymes and hormones are ongoing as a part of daily life, while these messengers orchestrate the coordinated Ca^{2+} signals.

To complete this story, great efforts are still needed. The detailed mechanism of biogenesis of these two messengers is still not completely understood. Do they totally depend on CD38 for synthesis? Do other catalytic systems exist? How will the topological paradox of CD38 be resolved? Is the nucleotide transporter system universal for mediating the cADPR/NAADP signaling? Does type-III CD38 contribute to the signaling as well? If so, how is it regulated? Besides these questions, additional issues about the targets of cADPR and NAADP need to be resolved as well. That the activation of RYRs by cADPR needs accessory proteins is generally accepted, but the identity of these proteins is still uncertain. For NAADP, recently published papers suggest that TPCs may not be the direct targets of NAADP, based on photoaffinity labeling using an analog

of NAADP [112,113]. Clearly, concerted effort is needed to further advance our understanding of these novel signaling messengers, an understanding that promises to have far-reaching consequences for the field of cell signaling in general.

Funding

This work was supported by the grants from the Council of Hong Kong (Nos. 769107, 768408, 769309 and 770610) and the National Natural Science Foundation of China/the Research Grants Council of Hong Kong (No. N_HKU 722/08).

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