**Ca²⁺ signalling: a new route to NAADP**

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NAADP (nicotinic acid–adenine dinucleotide phosphate) is a derivative of NADP (nicotinamide–adenine dinucleotide phosphate), which differs by the presence of a nicotinic acid instead of a nicotinamide moiety. This small structural difference makes NAADP one of the most powerful second messengers known, able to mobilize intracellular Ca²⁺ in a wide range of cellular models, ranging from invertebrates to mammals. Despite this, our understanding of NAADP homoeostasis, metabolism and physiological action is still limited. A new report by Vasudevan and colleagues in this issue of the *Biochemical Journal* provides important new data by describing a new synthetic activity in sperm cells which may turn out to represent the most physiologically relevant route to this second messenger.

Key words: ADP-ribosyl cyclase, calcium, nicotinic acid–adenine dinucleotide phosphate (NAADP), sea urchin, second messenger, sperm.

NAADP (nicotinic acid–adenine dinucleotide phosphate) is one of the most powerful second messengers known, able to mobilize intracellular Ca²⁺ in a wide range of cellular models, ranging from invertebrates to mammals [1]. It activates a Ca²⁺-release pathway distinct from those activated by IP₃ (inositol 1,4,5-trisphosphate) and cADPR (cADP-ribose) [2] and may engage a distinct non-ER (endoplasmic reticulum) Ca²⁺ pool. The latter appears to be acidic, and, in sea urchin egg homogenates, ‘reserve pool’ lysosome-like granules have been identified as a Ca²⁺ store which is sensitive only to NAADP [3]. Similarly, NAADP, as well as cADPR, but not IP₃, releases Ca²⁺ from a VAMP2 (vesicle-associated membrane protein 2)-positive compartment, which is likely to correspond to insulin granules in pancreatic β-cells [4].

NAADP has up to now been thought to be synthesized by one or more members of the ADP-ribosyl cyclase family, which includes a cyclase from *Aplysia californica* (the first characterized) [5] and the cell-surface antigens CD38 [6] and CD157 [7], in response to undefined signals. The reaction leading to the formation of NAADP is known as base-exchange, where, utilizing NADP (nicotinamide–adenine dinucleotide phosphate) as substrate and at acidic pH, the enzyme catalyses the exchange of nicotinamide with nicotinic acid [8] (Figure 1).

The above enzymes are defined as multifunctional because they are able to catalyse distinct reactions [9], i.e. both base-exchange (to generate NAADP), cyclization (to generate cADPR) (Figure 1) and product hydrolysis. These reactions appear to be differentially regulated, and show distinct responses to pH (cADPR is preferentially synthesized over NAADP at neutral pH, whereas the base-exchange reaction predominates at acidic pH [6]). Moreover, in pancreatic β-cells, millimolar concentrations of ATP inhibit the hydrolytic activity of CD38 towards cADPR [10], whereas Zn²⁺ ions are able to enhance CD38 cyclic activity [11]. In sea urchins, cGMP stimulates cADPR synthesis [12], whereas cAMP stimulates NAADP synthesis [13]. Finally, a recent study [14] suggests that there are enzymes other than CD38 in mammalian tissues that are able to produce NAADP via mechanisms different from the base-exchange reaction (deamination of NADP or phosphorylation of NAAD).

A new study by Vasudevan and colleagues [15] reported in this issue of the *Biochemical Journal* suggests the presence, in sea urchin sperm, of a new NAADP synthase. By comparing the conversion of NADP into NAADP in intact sperm with that in permeabilized sperm, the authors demonstrate that the enzyme (in common with members of the family of ADP-ribosyl cyclases) is situated largely on the plasma membrane and possesses an activity which is pH-dependent. The important finding of the new study is that the enzyme displays only base-exchange, and not cyclase, activity (Figure 1), suggesting that it may represent the ‘genuine’ NAADP synthetic enzyme, at least in sea urchin sperm. Secondly, the finding that the NAADP synthase is regulated by Ca²⁺ provides evidence for a new regulatory positive-feedback loop during physiological stimulation. Indeed, the ‘bell-shaped’ Ca²⁺ dose–response curve for the new activity may mean that the balance of NAADP synthesis and breakdown are likely to be under close control by cytosolic Ca²⁺ concentration. Thus, at least in mouse brain homogenates, NAADP is dephosphorylated to NAAD via a mechanism that is also Ca²⁺-dependent [16]. It also follows that, in response to stimuli which raise cytosolic Ca²⁺ through other means (e.g. glucose-induced depolarization in the case of β-cells), NAADP increases [17] may be as much a consequence as a cause of an initial Ca²⁺ rise. Nevertheless, NAADP-induced Ca²⁺ release may nonetheless serve as a trigger in this system to generate local Ca²⁺ increases, possibly close to secretory granules, through Ca²⁺-induced Ca²⁺ release [18].

The new study raises several important new perspectives. A conundrum surrounding the mechanisms of NAADP synthesis arises from the localization of the known synthetic enzymes. CD38, the only molecule thought previously to produce NAADP in mammalian tissues, is an ectoenzyme, with the catalytic domain situated at its C-terminus, in the extracellular space, where the acidic pH used to catalyse the base-exchange reaction is unlikely to pertain. In common with CD38, the localization of the new activity on the extracellular surface, leads to a ‘topological paradox’ (Figure 2). This localization obviously requires that a specific transport mechanism exists both for substrate (NADP) efflux and for product (NAADP) influx; of

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Figure 1 Members of the family of the ADP-ribosyl cyclases catalyse two main reactions

(A) Base-exchange reaction. Utilizing NADP as substrate and at acidic pH, the enzymes catalyse the exchange of nicotinamide with nicotinic acid. (B) Cyclization. NAD\(^+\) is converted into cADPR via cyclization. ARC, ADP-ribosyl cyclase.

Figure 2 Synthase localization

(A) The synthase catalytic domain is situated in the extracellular space and requires substrates to be transported outside the cell and product (NAADP) inside via transporters. A pH of 7 and a cytosolic Ca\(^{2+}\) concentration of 1 mM seem likely to suppress the base-exchange reaction at the cell surface. However, it is possible that Ca\(^{2+}\) action on the enzyme may occur only via a cytosolic domain, in which case activity may still be present in the face of high extracellular Ca\(^{2+}\). Fortunately, this possibility can be readily tested using intact sperm.

Although the study of Vasudevan and colleagues [15] is important, it is clear that much remains to be done. First, molecular identification of the NAADP synthetic enzyme activity is needed and should allow an assessment of its expression and overall contribution to NAADP synthesis in sperm and in other cell types: RNA interference or gene-knockout approaches in different species and cell types are likely to be illuminating. Studies are also required to explain how the activity is regulated in response to cellular stimulation. Such analyses, as well as complementary studies to identify the receptor(s) for NAADP at the molecular level [20], are eagerly awaited.

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