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Molecular characterization of a novel cell surface ADP-ribosyl cyclase from the sea urchin

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ABSTRACT

The sea urchin is an extensively used model system for the study of calcium signalling by the messenger molecules NAADP and cyclic ADP-ribose. Both are synthesized by ADP-ribosyl cyclases but our molecular understanding of these enzymes in the sea urchin is limited. We have recently reported the cloning of an extended family of sea urchin ADP-ribosyl cyclases and shown that one of these enzymes (SpARC1) is active within the endoplasmic reticulum lumen. These studies suggest that production of messengers is compartmentalized. Here we characterize the properties of SpARC2. SpARC2 catalyzed both NAADP and cyclic ADP-ribose production. Unusually, the NAD surrogate, NGD was a poor substrate. In contrast to SpARC1, heterologously expressed SpARC2 localized to the plasma membrane via a glycosylphosphatidylinositol (GPI)-anchor. Transcripts for SpARC2 were readily detectable in sea urchin eggs and a majority of the endogenous membrane bound activity was found to be GPI-anchored. Our data reveal striking differences in the properties of sea urchin ADP-ribosyl cyclases and provide further evidence that messenger production may occur outside of the cytosol.

1. Introduction

ADP-ribosyl cyclases constitute a family of multifunctional enzymes that can synthesize and metabolise a range of molecules involved in calcium homeostasis [1,2]. The first ADP-ribosyl cyclase to be cloned was a soluble form from *Aplysia* [3]. Two mammalian homologues, CD38 [4] and CD157 [5] have since been identified and more recently a homologue from *Schistosoma* [6]. CD38 has been the most extensively studied family member. This enzyme catalyses cyclic ADP-ribose production (by cyclization of NAD) and NAADP production (by base-exchange of the nicotinamide moiety in NADP with nicotinic acid) [1]. Both these molecules are potent calcium mobilizing messengers [7]. CD38 can also hydrolyze these molecules [4,8] and catalyze novel reactions that produce adenine homodinucleotides from cyclic ADP-ribose [9]. The latter modulates cytosolic calcium levels through distinct mechanisms. The major activity of CD38 is the hydrolysis of NAD directly to ADP-ribose [4]. This nucleotide stimulates calcium influx via activation of TRPM2 channels [10]. That calcium signals regulate a whole range of cellular processes underscores the physiological importance of these ubiquitous enzymes. Indeed, ADP-ribosyl cyclases have been implicated in cellular processes ranging from temperature stress in sponges [11] to social behaviour in mice [12].

The presence of signal sequences in all ADP-ribosyl cyclase family members renders the catalytic site of the enzyme within the extracellular/lumenal face. Indeed, the presence of multiple disulfide bonds limits their location to oxidizing environments. Accordingly, CD38, CD157 and the *Schistosoma* enzyme are predominantly located on the plasma membrane, the latter two via glycosylphosphatidylinositol (GPI) anchors [1,2]. This location poses a topological conundrum for enzymes that produce messenger molecules active in the cytosol. Nevertheless, the use of the CD38 knockout mouse has clearly implicated the role of this ADP-ribosyl cyclase in generating cyclic ADP-ribose *in vivo* [13]. This so called "topology paradox" [14] might indicate that messenger synthesis occurs in the extracellular space followed by transport of products into cells [15]. Alternatively, synthesis may occur in a luminal environment following internalization of surface enzymes [16]. Indeed, there are many reports for an intracellular location of CD38 [1,2] although topology constraints still necessitate mechanisms for transport of substrates into, and release from, CD38 expressing organelles.

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Table 1
Primer sequences

Primer	Sequence
SpARC2 1F	5′-CACCAGATCTATGATGAATCTTCTCCGCACG-3′
SpARC2 1R	5′-GCTCTAGAGTCAATCAATTTTGCAACCATTTAGCA-3′
SpARC2 2F	5′-GAGCAAAAGCTCATTTCTGAAGAGGACTTGGGACCAGGAACCACATGG-3′
SpARC2 2R	5′-CAAGTCCTCTTCAGAAATGAGCTTTTGCTCTGGCAAGGTATAGGCAACAGT-3′
SpARC2 3F	5′-CACCGAATTCAGGACCAGGAACCACATGG
SpARC2 3R	5′-ACCCAATCGATGTGGCAAGGTATAGGCAACAGT-3′
SpARC2 4R	5′-GCTCTAGAGTCATTCCTGCCTGCTGTATGG-3′
mRFP 1F	5′-CACCATCGATTTATGGCCTCCTCCGAGG-3′
mRFP 1R	5′-CCTTGAATTCAAGGCGCCGGTGGAGT-3′
SpARC1 1F	5′-CACCAGATCTATGGGCATCTACACCATATTCA-3′
SpARC1 1R	5′-GCTCTAGAGTTATAGGGTAGTAGATATTGTATTCCAACC-3′
SpARC2 5F	5′-GCGATGTCAACTGTGATGCT-3′
SpARC2 5R	5′-CTCACAAGTGTTGCTGAGACC-3′

Introduced restriction sites are underlined.

ADP-ribosyl cyclase activity was first discovered in eggs from the sea urchin [17] and much has been learned using this model organism regarding the mechanism of action of cyclic ADP-ribose and NAADP [18,19]. Although multiple activities have been characterized in cell homogenates [20,21], molecular details of the synthetic machinery have been conspicuously lacking. We recently determined the primary structures of three sea urchin ADP-ribosyl cyclases [22]. We provided evidence that SpARC1, the first member of this expanded family, is localized to the endoplasmic reticulum lumen and can gain access to extra-luminal substrate. These data provide molecular evidence that messenger synthesis may be compartmentalized. In the present study, we report properties of SpARC2. We demonstrate that that SpARC2 has unusual substrate preference and is a GPI-anchored plasma membrane protein. Additionally, we find that a substantial fraction of ADP-ribosyl cyclase activity in sea urchin eggs is GPI-anchored. Our data provide further evidence that messenger production may occur in extra-cytosolic compartments.

2. Experimental procedures

2.1. Generation of expression constructs

The full-length coding sequence of SpARC2 was amplified from a *Strongylocentrotus purpuratus* ovary cDNA library, kindly provided by Professor Gary Wessel (Brown University, USA), using the polymerase chain reaction (PCR) and primers SpARC2 1F+1R (Table 1). The product, which included the ATG codon immediately preceding the predicted start codon, was then cloned into pCS2+ (http://sitemaker.umich.edu/dlturner.vectors/home) at the BamHI/XbaI sites, to generate an expression construct encoding for SpARC2 untagged (Fig. 1B). Constructs for *myc* and monomeric RFP (mRFP)-tagged SpARC2 (Fig. 1B) were generated by inserting the appropriate tag

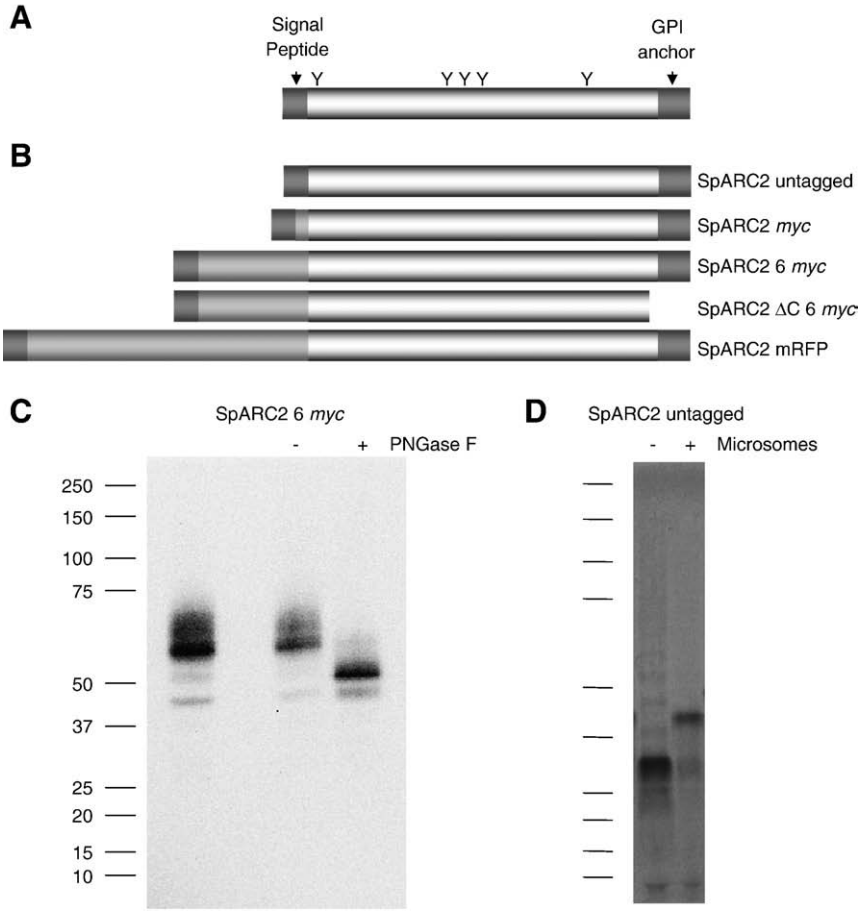

Fig. 1. Predicted structure of SpARC2 and heterologous expression. A, Schematic representation of SpARC2. Positions of the putative signal peptide, glycosylation sites (Y) and glycosylphosphatidylinositol anchor are indicated. B, Expression constructs used throughout this study. The introduced tags (light shading) are indicated. C, Western blot of homogenates prepared from *Xenopus* embryos expressing SpARC2 6 *myc*. The right hand lanes show the effect of mock (−) and PNGase F (+) treatment on migration of SpARC2 6 *myc*. Blots were probed with an anti-*myc* antibody. D, *In vitro* translation of SpARC2 untagged in the absence (−) and presence (+) of microsomes.