Sperm Biology

Is PAWP the "real" sperm factor?

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Mammalian embryo development is initiated by intracellular Ca^{2+} oscillations that result in oocyte activation following gamete membrane fusion. It is widely believed that oocyte Ca^{2+} oscillations are triggered by a sperm-specific protein, phospholipase C-zeta (PLCζ) that activates InsP3 production leading to repetitive Ca^{2+} release from intracellular stores. However, a recent report in the *FASEB Journal* by Aarabi *et al.* challenges this view by proposing postacrosomal WW domain-binding protein (PAWP) as another sperm-derived protein that can also initiate Ca^{2+} oscillations and zygotic development at fertilization. Here we discuss these new findings and examine the evidence suggesting PAWP as the "real" sperm factor.

At fertilization, the first event following the fusion of sperm and oocyte membranes is a series of transient rises in the intracellular-free Ca^{2+} concentration, termed Ca^{2+} oscillations.[1–3] Over the past decade, mounting experimental and clinical evidence has been congruent with the hypothesis that the sperm factor responsible for the initiation of Ca^{2+} oscillations during mammalian fertilization is a testis-specific isoform of PLC-zeta (PLCζ).[4–8] Since the discovery of PLCζ in 2002, many research laboratories across the world (**Table 1**) have reported experimental evidence compatible with the proposition that PLC, termed PLCζ is the sperm factor that causes Ca^{2+} oscillations at fertilization. Based upon numerous complementary studies, the current understanding of the molecular mechanism of PLCζ action in mammalian oocytes is summarized in **Figure 1** (left side). Upon sperm-oocyte membrane fusion, PLCζ diffuses from the sperm head into the oocyte cytoplasm and hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) located in an intracellular vesicle compartment. The resulting liberation of $InsP_3$ stimulates opening of the $InsP_3R$ Ca^{2+} release channel on the endoplasmic reticulum that results in Ca^{2+} oscillations, oocyte activation and embryo development.[3]

The recent FASEB Journal paper by Aarabi *et al.*[9] reports that PAWP, a sperm head protein that exclusively resides in the postacrosomal sheath region of the perinuclear theca, is able to produce Ca^{2+} oscillations and pronuclear formation in human and mouse oocytes similar to what is observed during intracytoplasmic sperm injection. This group previously identified PAWP[10] as an alkaline-extractable protein with sequence homology to the N-terminal half of WW domain-binding protein 2, while the C-terminal half is rich in proline residues. Aarabi *et al.*[9] also report that sperm-induced Ca^{2+} oscillations are blocked by co-injection of a competitive peptide inhibitor, derived from the WWI domain-binding motif of PAWP. This implies there is a requirement for PAWP binding to an oocyte-derived protein for successful fertilization to occur (**Figure 1**, right side).

The recent indication of PAWP-induced Ca^{2+} signalling[9] in oocytes comes 7 years after this group's initial data showing that PAWP promotes meiotic resumption and pronuclear development during fertilization.[10] Based on their studies, microinjection of PAWP protein into porcine, bovine, macaque, and Xenopus oocytes resulted in pronuclear formation, an indicative event of successful oocyte activation.[10] However, to date, no other research groups have independently verified PAWP's ability to activate oocytes and/or cause Ca^{2+} oscillations. Recently, we have investigated whether mouse PAWP can initiate Ca^{2+} oscillations and oocyte activation in the mouse.[11] We microinjected into mouse oocytes the recombinant mouse PAWP protein, or the complementary RNA encoding either untagged PAWP, or YFP-PAWP, or PAWP-luciferase, but we consistently failed to observe any Ca^{2+} increases. In addition, PAWP was unable to hydrolyse PIP_2 *in vitro* and also did not act as a generic activator of PLC activity.[11] To the best of our knowledge, this is the first attempt to independently confirm the key findings on PAWP by the Oko group, but we could not verify that PAWP has any ability to mobilize intracellular Ca^{2+} or activate mouse oocytes.

Aarabi *et al.*[9] also state that PAWP and an oocyte-derived WWI domain protein substrate is required for successful fertilization, because sperm-induced Ca^{2+} oscillations were blocked by co-injection of a 16-amino acid proline-rich peptide, derived from the PAWP WWI domain binding motif. Interestingly, the negative control peptide that was used had only a single amino acid substitution (Tyr/Phe) and this did not affect the sperm-induced Ca^{2+} oscillations in oocytes.

Table 1: Academic institutions where independent research laboratories (as defined by the corresponding author's address) have published experimental evidence up to September 22, 2014, in peer-reviewed journals, that support either PAWP (left) or PLCζ (right), as the mammalian sperm factor responsible for initiating calcium oscillations and oocyte activation at fertilization

PAWP	*PLCζ*
Queen's University, Canada	Cardiff University, UK
University of Missouri, USA	University of Massachusetts-Amherst, USA
Tabriz University, Iran	University of Oxford, UK
	Ghent University Hospital, Belgium
	"N.C.S.R." Demokritos, Greece
	Stony Brook University, USA
	Michigan State University, USA
	Cornell University, USA
	Tokyo Women's Medical University, Japan
	Azabu University, Japan
	RIKEN Center Developmental Biology, Japan
	Monash University, Australia
	Academy of Agricultural Science, China
	CHA University, Korea
	Royan Institute Reproductive Biomedicine, Iran
	São Paulo State University, Brazil

PAWP: postacrosomal WW-domain binding protein; PLCζ: phospholipase C zeta

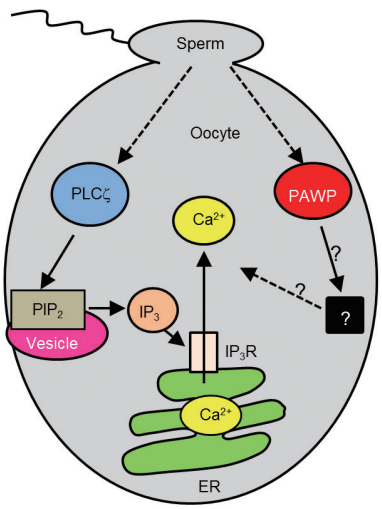

Figure 1: Schematic representation of known mechanisms of action of the sperm proteins, PLCζ (left side) and PAWP (right side) in mammalian oocytes. ER: endoplasmic reticulum; PIP_2: phosphatidylinositol 4,5-bisphosphate; IP_3: inositol 1,4,5-trisphosphate; PAWP: postacrosomal WW domain-binding protein; PLCζ: phospholipase C zeta.

The results with these PAWP peptides has led to the hypothesis that PAWP mediates its effects in oocytes via interaction with other proteins, such as the yes-associated protein, that may then lead to activation of PLCγ. However, previous studies using SH2 domain-derived peptides have suggested that PLCγ does not mediate Ca^{2+} oscillations in fertilizing mouse oocytes.[12] Hence, if PAWP mediates any potential effects via PLCγ, then its precise role in physiological activation during fertilization remains to be clarified. As with the data they obtained using the recombinant PAWP protein, it will be important for other groups to independently investigate these specific claims for the potent inhibitory effects of these PAWP-derived proline-rich peptides, since these claims have important implications for our understanding of the mechanism of Ca^{2+} release at fertilization.

It is worthwhile to reflect that over the last few decades there have been various sperm-derived molecules implicated in activating the oocyte by causing Ca^{2+} release. These previous "sperm factor" candidates include a 33 kDa protein,[13] nitric oxide,[14] and tr-kit, a truncated form of the c-kit receptor.[15] None of these molecules stood the test of time, mainly because subsequent research either could not validate or else did not build upon, the original data. In contrast, when PLCζ was first shown to cause Ca^{2+} oscillations in mouse oocytes, two independent verification and extensions of the original findings were reported within 2 years.[6,16] In the following decade, many other groups have confirmed that PLCζ causes Ca^{2+} oscillations in oocytes from a range of different species (**Table 1**).[3] Interestingly, immunolocalization analysis has indicated that PLCζ, like PAWP, is also present in the perinuclear matrix of the sperm.[16] At present, unfortunately, there remains no published data from mouse "knockout" models for either PLCζ or PAWP. The availability of such mice should provide the conclusive evidence of a direct physiological role for these mammalian sperm proteins in the Ca^{2+} signaling that is essential for oocyte activation.

So despite the intriguing new data presented in the paper by Aarabi *et al.*[9] there is a need for further independent verification of these important results so that PAWP can then also be considered a candidate for the "real" sperm factor, which mobilizes the physiological Ca^{2+} signal that triggers oocyte activation and early embryo development.

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