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Review

Aquaporins in sperm osmoadaptation: an emerging role for volume regulation

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Upon ejaculation, mammalian sperm experience a natural osmotic decrease during male to female reproductive tract transition. This hypo-osmotic exposure not only activates sperm motility, but also poses potential harm to sperm structure and function by inducing unwanted cell swelling. In this physiological context, regulatory volume decrease (RVD) is the major mechanism that protects cells from detrimental swelling, and is essential to sperm survival and normal function. Aquaporins are selective water channels that enable rapid water transport across cell membranes. Aquaporins have been implicated in sperm osmoregulation. Recent discoveries show that Aquaporin-3 (AQP3), a water channel protein, is localized in sperm tail membranes and that AQP3 mutant sperm show defects in volume regulation and excessive cell swelling upon physiological hypotonic stress in the female reproductive tract, thereby highlighting the importance of AQP3 in the postcopulatory sperm RVD process. In this paper, we discuss current knowledge, remaining questions and hypotheses about the function and mechanismic basis of aquaporins for volume regulation in sperm and other cell types.

Keywords: aquaporins; sperm; water permeability; regulatory volume decrease; osmosensing/mechanosensing; tetrameric structure

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Efficient sperm volume regulation is a prerequisite for normal sperm function

In most mammalian species studied, the journey of sperm from the male to the female reproductive tract experience a natural osmotic decrease[1], an evolutionary vestige from freshwater fish species[2]. Before ejaculation, the mammalian sperm are quiescent in the relatively hypertonic male reproductive tract with no or very low motility. Upon copulation, the sperm enter into the relatively hypotonic female reproductive tract and quickly show motility activation[3, 4], indicating that osmotic changes are beneficial for initial sperm motility activation. However, postcopulatory hypotonic stress also has a negative effects as it induces osmotic cell swelling, which if uncontrolled, can be detrimental to sperm function and survival[5]. Mammalian sperm have evolved to effectively reduce the negative impact of hypotonic cell swelling by means of regulatory volume decrease (RVD), which was proposed to involve efficient volume regulation driven by active solute transport and rapid transmembrane water movement[6].

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Functional importance of aquaporins in sperm volume regulation: emerging evidence from AQP3 knockout mice

In the early 1970s, it was demonstrated that the water permeability coefficient of bull spermatozoa was quite high, about four times greater than that of bovine erythrocytes and between ten and thirty times greater than that of artificial bimolecular lipid membranes[7]. According to these observations, the author made an insightful conclusion that the chief route for the passage of water through the sperm membrane must be via "pores"[7]. Similar high water permeability coefficients have been discovered in other mammalian species, including humans[8, 9].

In the past two decades, the understanding of the movement of water through cell membranes has been greatly advanced by the discovery of aquaporins, a family of water-specific membrane channel proteins[10]. Recently, it was proposed that aquaporins might be active players in sperm volume-regulatory water flux[11]. Indeed, two aquaporins (AQP7 and 8) were first cloned from rat testes and identified by staining in murine sperm tails[12–14]. Aquaporin-11 (AQP11) has also been found in the end piece of rat sperm[15] (although some controversies exist due to variable antibody specificity; for a more detailed description, see the comprehensive review[14]). However, genetic deletion of AQP7 and 8 in mice did not result in obvious abnormalities in sperm morphology and function[11, 16, 17],

suggesting that these AQPs are not essential for mouse sperm function or that they can be functionally substituted by other aquaporin members.

After a previous study identified Aquaporin-3 (AQP3) expression in mouse testes[18], we demonstrated that AQP3 was present in both mouse and human sperm and was located in the plasma membrane of the principle piece of flagellum[3] (Figure 1A). Further functional studies using AQP3 knockout mice have shown that upon exposure to physiological hypotonic stress, AQP3 mutant sperm show normal initial motility but display increased vulnerability to hypotonic cell swelling characterized by increased tail bending after entering the uterus[3]. The observed sperm defect was due to impaired cell volume regulation and progressive cell swelling in response to physiological hypotonic stress, as revealed by sperm volume detection using flow cytometry in a population-based manner and by time-lapse imaging of individual sperm[3]. The tail deformation hampers normal sperm migration into the oviduct, resulting in impaired fertilization and reduced male fertility[3]. These results provided direct evidence that AQP3 was actively involved in mouse sperm volume regulation during physiological hypotonic stress by protecting sperm from excess cell swelling and, therefore, optimizing postcopulatory sperm behavior.

Notably, it has also been demonstrated that, compared to mouse sperm, human sperm show a strikingly similar pattern of AQP3 localization[3], thus providing the intriguing possibility of a similar role for AQP3 in human sperm. On the other hand, we failed to detect AQP3 expression in rat sperm, which is consistent with previous reports[19]. Indeed, such species-specific expression of AQP3 has been observed in other tissues. For example, AQP3 is expressed in human and rat erythrocytes but not in mouse erythrocytes[20, 21]. The discrepancies in AQP3 expression patterns between closely related species such as mice and rats suggest a dynamic selection of AQP3 expression during evolution.

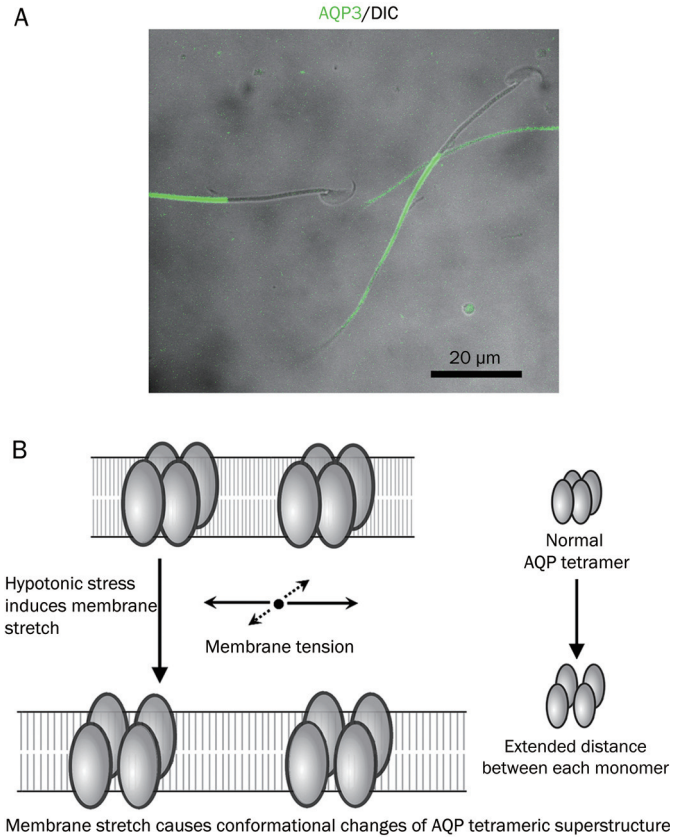

Figure 1. (A) AQP3 localization in mouse sperm. (B) A hypothesized model showing how the AQP tetrameric structure could be involved in cellular mechanosensing during hypotonic stress-induced cell swelling.

How do AQP proteins mediate cell RVD during hypotonic stress?

Despite the observation that AQP3 is important for normal sperm RVD during hypotonic exposure[3] and the increasing body of evidence that several members of AQPs (AQP1, 2, 3, 4, and 5) are actively involved in RVD of diverse cell types[22–26], the molecular mechanisms by which AQPs take part in RVD are hard to explain using the "simple permeability" theory, which considers aquaporins as inert pores that simply increase the osmotic permeability of plasma membranes. Under such a "simple permeability" model, RVD begins with a hypotonic stress-induced water influx, followed by active solute transport that enables osmolyte efflux and provides the driving force for water to exit[27], and the participation of AQPs in RVD is merely to facilitate the time it takes to reach osmotic equilibrium. When applying such a model in the AQP3 mutant sperm, since both water influx and efflux were supposed to be equally diminished, the progressive sperm cell swelling should not have been observed.

Given such a dilemma, it is our belief that AQP3-dependent water permeability in sperm, if any, is not the primary contribution to sperm RVD under hypotonic stress. Alternatively, we hypothesize that AQP3 may function as a part of the membrane osmosensing/mechanosensing system for the initial sensing of cell swelling and therefore, may "trigger" subsequent RVD events such as solute transport and cytoskeleton reconstruction. This hypothesis is supported by the fact that AQP3 is strongly expressed in many organs, such as the bladder, trachea and esophagus, and in olfactory cells[18, 28, 29], where they seem to have no obvious requirements for rapid water movement but need sensitive perception of tension, shear stress and so on. Moreover, this hypothesis is supported by the recent discovery that AQP5 is actively involved in salivary gland cell RVD by coordinating with TRPV4, a volume sensitive calcium channel, to concertedly regulate cell volume under hypotonic stimulation[23]. This provides the first mechanistic evidence that AQPs are actively involved in upstream events of cell volume sensing through interaction with other volume-sensitive ion channels. Interestingly, the scenario of volume regulation by AQP5/TRPV4 interaction has recently been expanded for AQP4. As demonstrated in mouse astrocytes, the TRPV4/AQP4 complex plays an important role in the initiation of RVD similar to that of the TRPV4/AQP5 complex in salivary gland cells[25]. In this regard, it would

not be hard to imagine that AQP3 might also form molecular complexes with other ion channels to mediate sperm RVD. Such candidate molecules may involve the volume sensitive chloride channel CLC-3[30], which has been observed in mammalian sperm and has been implicated in sperm volume regulation[31, 32].

Despite the accumulating evidence, a most basic question remains for the involvement of AQPs in RVD: As water channel proteins, by what structural basis do AQPs play a role in osmosensing/mechanosensing? We propose that the answer to this question might reside in the homotetrameric structure of aquaporins, as revealed by molecular crystal structure analysis[10]. Although it has been established that the water permeability characteristics of AQPs rely on the pores of each monomer, the evolutionary driving force for AQPs to form a tetramer is not understood[10]. It is hypothesized that the homotetrameric nature of aquaporins could provide a structural base to sense membrane stretching during hypotonic stress-induced cell swelling. As shown in Figure 1B, cell swelling in response to hypotonic stress would increase membrane tension and might result in an extended distance between each AQP monomer and cause conformational changes of the homotetramer. Such changes in molecular structure may initiate downstream signaling cascades for RVD events. This model would be particularly suitable in cases when AQPs form functional complexes with other mechanosensors (such as TRPV4[23, 25]) or directly interact with cytoskeletal components such as actin filaments (AQP2 has been shown to interact directly with actin[33]). However, to stringently test such a hypothesis, it would be necessary to create a mutant AQP protein that could not form a tetrameric structure while maintaining the water permeability of each AQP monomer.

There is also a remote possibility that under processes such as sperm RVD under hypotonic stress, AQP3 functions as a unidirectional water channel allowing only water efflux, while other sperm AQPs, such as AQP7 and 8, only allow water influx. Indeed, recent findings have demonstrated that a formate transporter shows an AQP-like channel structure[34, 35], thus lending support to the radical notion that an aquaporin structure may have the potential to function as a unidirectional water transporter in cooperation with other molecules, at least under certain circumstances.

Conclusion

In summary, the emerging evidence for AQPs in cell volume regulation, including AQP3 in sperm, can not be fully explained by considering AQPs as inert pores simply for water permeability. These phenomena provide future directions for a new round of AQP research focused on AQPs with more fundamental roles as general regulators, such as osmosensors/mechanosensors, possibly with novel mechanisms.

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