J. Microbiol. Biotechnol. (2005), **15**(3), 665–671

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Cell Signaling Mechanisms of Sperm Motility in Aquatic Species

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Received: October 25, 2004
Accepted: December 21, 2004

Abstract Initiation and activation of sperm motility are prerequisite processes for the contact and fusion of male and female gametes at fertilization. The phenomena are under the regulation of cAMP and Ca^{2+} in vertebrates and invertebrates. Mammalian sperm requires Ca^{2+} and cAMP for the activation of sperm motility. Cell signaling for the initiation and activation of sperm motility in the ascidians and salmonid fishes has drawn much attention. In the ascidians, the sperm-activating and attracting factors from unfertilized egg require extracellular Ca^{2+} for activating sperm motility and eliciting chemotactic behavior toward the egg. On the other hand, the cAMP-dependent phosphorylation of protein is essential for the initiation of sperm motility in salmonid fishes. A decrease of the environmental K^+ concentration surrounding the spawned sperm causes K^+ efflux and Ca^{2+} influx through the specific K^+ channel and dihydropyridine-sensitive L-/T-type Ca^{2+} channel, respectively, thereby leading to the membrane hyperpolarization. The membrane hyperpolarization induces synthesis of cAMP, which triggers further cell signaling processes, such as cAMP-dependent protein phosphorylation, to initiate sperm motility in salmonid fishes. This article reviews the studies on the physiological mechanisms of sperm motility and its cell signaling in aquatic species.

Key words: Sperm, motility, cell signaling, salmonid, teleost

The state of spermatozoan motility can be generally divided into four steps during its life history. The first step is the process of spermatogenesis, in which spermatogonia proliferate, grow, and metamorphose into morphologically specialized cells, spermatozoa. In this process, cells are fixed in the testes with supporting cells such as Sertoli's cell and cyst cell, and they are immotile in the testes. During spermiation, spermatozoa are released from supporting cells and discharged from the testes, and then the second step occurs: they acquire the potential for motility as spermatozoa reach the vas deferens. Even though the sperm cells acquire the potential to move, some factors included in seminal plasma suppress sperm motility. Thus, they remain immotile in the male reproductive organ. In the third step, the initiation of sperm motility occurs after spermatozoa are spawned or ejaculated. The fourth step involves motility activation or chemotactic behavior of spermatozoa, which occurs during their approach to the egg before fertilization.

The regulation of motility during these processes has been the subject of several studies. In this paper, recent studies on the initiation and activation phenomena of sperm motility in several marine species are reviewed, and its mechanisms are suggested.

Factors Initiating Sperm Motility in Internal Fertilization Species

Studies on the mechanisms of the initiation and activation of sperm motility at spawning are very useful in understanding not only the mechanism for flagellar movement, but also the intracellular signal transduction system in general. Processes of the initiation and activation of sperm motility are different from each other, depending on the types of fertilization, viz. external fertilization or internal fertilization. In external fertilization species, the spermatozoa obtain motility at the time of spawning in the aquatic environment; for example, seawater. In internal fertilization species, the initiation of sperm motility occurs upon mixing with the seminal plasma at ejaculation. However, the major factors for the initiation and activation of motility in the external and internal fertilization species are included in the seminal plasma and external environment surrounding the spawned or ejaculated sperm. In salmonid fishes, transmembrane cell signaling for the initiation of sperm motility is controlled by the changes in environmental ionic conditions at spawning from the male reproductive tract to the spawning

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ground, which is fresh water in the external fertilization species.

In the sperm of mammals, internal fertilization species, HCO_3^- and Ca^{2+} are well known as the factors for the initiation and activation of sperm motility. It has been reported that external Ca^{2+} is required for the activation of motility in bovine, hamster, and rat sperm [6, 7, 51, 52]. However, the sperm of the mouse, rabbit, and humans are motile upon release from the epididymis, even in the absence of exogeneous Ca^{2+} [31, 51, 66]. On the other hand, cyclic nucleotides, particularly cAMP, may be the universal factor for the activation of mammalian sperm motility [42, 52]. HCO_3^- stimulates the synthesis of cAMP from ATP via activation of adenylyl cyclase, resulting in the activation of porcine [57, 68] and hamster sperm motility [61, 63]. Tash and his colleagues [72, 73] found a detergent-soluble phosphoprotein, named axokinin, which was identified as the regulatory subunit type II (R II) of cAMP-dependent protein kinase (PKA), for activation of the motility of dog sperm. Furthermore, it was revealed that cAMP-dependent phosphorylation of 36 and 65 kDa proteins regulates the velocity of microtubule sliding in the sperm of the hamster [64] and mouse [61, 62], respectively. In these species, tyrosine phosphorylated protein of 80 kDa is considered as a hyperactivated-motility producing protein [65]. In the fowl, the activation of sperm depends on the changes in temperature, and calmodulin-dependent kinase is necessary for its regulation. Furthermore, motility of the demembranated fowl sperm was inhibited by myosin light-chain kinase inhibitor and phosphoprotein phosphatase I, indicating that the sperm motility in fowl is regulated by both temperature and protein phosphorylation [2–4].

Factors Initiating Sperm Motility in External Fertilization Species

In the sea urchin, a typical external fertilization species, Gray [29] first studied the role of environmental factor for the initiation of sperm motility. He described that sperm motility is initiated at spawning in the seawater by mechanical dilution, whereby each spermatozoon becomes surrounded by increased free space for movement. Rothchild [60] extended the hypothesis to include the effects of gas tension on the initiation of sperm motility. In his hypothesis, changes in the environment surrounding the sperm from CO_2-rich anaerobic testis to aerobic O_2-rich seawater cause the increase in respiration of sperm at midpiece mitochondria, resulting in supplement of the energy for sperm motility. Johnson *et al.* [35] then demonstrated that CO_2 suppresses sperm metabolism in the seminal plasma. Upon spawning, a decrease in CO_2 of the sperm environment induces β-oxidation of lipids in the plasma membrane of sperm to supply the energy source. In the downstream of the cell signaling by the external factors, the effect of ion fluxes has been recognized as being the transmembrane cell signal for the initiation of sperm motility. Nishioka and Cross [53] demonstrated that the efflux of H^+ and influx of Na^+ across the Na^+/H^+ antiporter cause the initiation of sperm motility. Tombes and Shapiro [74] further demonstrated that the intracellular alkalization activates dynein ATPase to initiate sperm motility, and the resulting consumption of ATP causes the beginning of high energy transport for sperm motility [74].

On the other hand, some factors derived from female gametes for the initiation and activation of sperm motility were found in some marine invertebrate species, such as sea urchin and annelids. In the sea urchin such as arbacia, a decapeptide, named speract, was purified from egg-jelly. The cell signaling factors for the initiation of sperm motility, which include cyclic nucleotides, cyclic nucleotide-gated K^+ channel, hyperpolarization of the plasma membrane [5, 21, 22, 26], alkalization of intracellular pH, and increase in intracellular Ca^{2+} level [28], have been proposed. Furthermore, it was suggested that the hyperpolarization activates adenylyl cyclase, and that cooperation of the enzyme and the increased Ca^{2+} cause synthesis of cAMP [8, 22]. The principal intracellular target for cAMP in the cells is PKA, which phosphorylates proteins and regulates cell functions. Ishiguro *et al.* [33] have shown that cAMP is a prerequisite for the activation of demembranated sea urchin sperm, and they identified the protein components with PKA activity. In addition to the PKA, other components are considered to be required for the cAMP-dependent reactivation of the demembranated sperm.

Egg-Derived Factors to Induce Both Activation and Chemotaxis in Ascidian Sperm

The cell signaling mechanisms for the activation of motility and the attraction of sperm have been investigated in the ascidians, such as *Ciona intestinalis* and *C. savignyi*. The binding of sperm-activating and sperm-attracting factor (SAAF), derived from the unfertilized egg, to the sperm activates K^+ channels to increase K^+ permeability of the sperm plasma membrane, resulting in membrane hyperpolarization. In turn, the hyperpolarization of the membrane potential activates the adenylyl cyclase and elevates the cAMP level in the sperm cytoplasm [34]. Cyclic AMP activates PKA and phosphorylates a 26 kDa protein and 21 kDa dynein light chain [54]. The phosphorylation of these proteins triggers the final step of the SAAF-induced activation of sperm motility.

In the external fertilization species of vertebrates, several egg-derived factors for the initiation and activation of sperm motility have also been reported in the teleost fishes. The sperm-activating substance was found in the coelomic fluid of the female rainbow trout [77]. Amanze and Iyenger [1] reported that the sperm of the rosy barb exhibited searching activity for the micropylar entrance of the egg. These results suggest that the spermatozoa could be fully

activated by the sperm-activating substance released from the egg to help spermatozoa reach the plasma membrane at fertilization. The Pacific herring is unique among marine teleosts in regards to sperm motility: Sperm are immotile or slightly motile in the hypertonic seawater, and only sperm that are close to eggs become actively motile, suggesting that some substances around the egg cause the the initiation and activation of sperm motility [50]. The substance was purified and named as the herring sperm activating proteins, HSAPs, by Oda *et al.* [56]. Identification of the molecular structure of the HSAPs by cDNA cloning revealed that the HSAPs are homologous to the trypsin inhibitors [55]. Furthermore, Yoshida [76] found several proteins with binding capacity to HSAPs. Among them, prolyl endopeptidase in the sperm surface was proposed as a receptor. On the other hand, Yanagimachi *et al.* [75] found another sperm activating factor that was tightly bound to the egg surface, and it was not easily diffusible into the seawater. Pillai *et al.* [58] purified this protein with a molecular size of 105 kDa and named it sperm motility initiation factor (SMIF). The factor is localized around the micropyle area of the unfertilized mature eggs [30, 75]. These suggested that sperm activated with HSAPs are guided into the micropyle by the effect of SMIF, resulting in the completion of fertilization in the herring. Both HSAPs and SMIF depolarize the sperm plasma membrane and cause influx of extracellular Ca^{2+}.

Role of Environmental Ionic and Osmotic Conditions to Induce Sperm Motility in Teleost Fishes

The changes in environmental ionic and osmotic conditions surrounding sperm are the factors, belonging to the category other than the egg-derived factors, for the regulation of sperm motility [49]. The role of the environmental factors for the initiation of sperm motility in teleost fishes was characterized by Morisawa and his colleagues. They found the physiological factors that can act as an on-off switch of sperm motility in the group of teleost fish [48, 49]. In marine and freshwater teleosts, spermatozoa are activated when they are exposed to the changes in osmolarity surrounding the sperm at spawning [48, 49]. Sperm of puffer fish [49], sea bass [9], black sea bream [18], marbled sole [40], gray mullet [17, 19], halibut [10], and Atlantic croaker [24] initiate motility when they are suspended into hyperosmotic water. On the other hand, sperm of freshwater cyprinid fish [49], goldfish [49], common carp [9, 41], zebra fish [69], and pejerrey [67] initiate motility when they are suspended into hypoosmotic water. Takai and Morisawa [69] demonstrated that change in intracellular K^+ concentration directly regulates sperm motility via change in external osmolality, in marine and freshwater teleosts. Extracellular divalent cation, Ca^{2+}, is involved in the signaling cascade for the initiation of sperm motility in teleosts of osmolality-dependent and K^+-decrease-dependent species. In the common carp, sperm motility is initiated with extracellular Ca^{2+} and thus regulated by dihydropyridine or peptide types of Ca^{2+} channel blockers [41].

K^+-Triggered Transmembrane Cell Signaling in Salmonid Fish Sperm

The intracellular signaling for the activation of sperm motility in teleosts has been well investigated in salmonid fishes. In these species, high concentration of K^+ in the seminal plasma suppresses the sperm motility in the male reproductive tract. Decrease in K^+ surrounding the sperm induces synthesis of cAMP [46], and the cAMP [47] activates PKA. The phosphorylation of the outer arm dynein light chain [32] and activation of tyrosine kinase to phosphorylate the 15 kDa protein [45] trigger the final step of the initiation of flagellar movement. Despite the accumulated knowledge on the intracellular cell signaling for the initiation of salmonid sperm motility, the transmembrane cell signaling has been poorly investigated. Participation of membrane hyperpolarization [12, 27, 70, 71], efflux of K^+, and influx of Ca^{2+} [23, 70] have been suggested so far, whereas the transmembrane cell signaling cascade, i.e., the relationship among K^+ channels, Ca^{2+} channels, membrane potentials, and cAMP synthesis, remains obscure.

Membrane hyperpolarization through a kind of voltage-dependent K^+ channel causes cAMP synthesis, thus leading to the initiation of sperm motility in salmonid fishes such as rainbow trout and steelhead trout [37, 39]. The Ca^{2+} influx through a kind of dihydropyridine sensitive L-/T-type Ca^{2+} channel may participate in the membrane hyperpolarization and synthesis of cAMP, which is the intracellular trigger for the initiation of sperm motility [38, 39]. Calmodulin, a calcium-binding protein, has been found to be an activator of cyclic nucleotide phosphodiesterase [20, 36]. This protein acts as an intracellular receptor for Ca^{2+} and plays a wide variety of roles in the cell signaling of eukaryotic cells [44], i.e., cyclic nucleotide metabolism, glycogen metabolism [25], and cell cycle [16]. In regard to cell motility, Ca^{2+}/calmodulin is required for the optimal activity of myosin light-chain kinase to phosphorylate the 20 kDa light-chain of myosin [59]. Ca^{2+}/calmodulin also plays a significant role in polymerization and depolarization of tubulin in the formation and degradation of mitotic spindle in the process of mitotic cell division [43]. Calmodulin may play a role in the regulations of flagellar motility as a Ca^{2+} sensor to change the flagellar bending pattern from symmetric to asymmetric wave and *vice versa*, according to the intracellular Ca^{2+} concentration in sea urchins [14, 15]. In sea urchins, purified adenylyl cyclase has an affinity to calmodulin [13]. It has also been reported that calmodulin activates ATPase of dynein chain in the cilia of *Tetrahymena pyriformis* [11]. In the ascidians, SAAF from the egg causes Ca^{2+} entry, and the Ca^{2+} activates calmodulin and calmodulin kinase II, leading to hyperpolarization of the

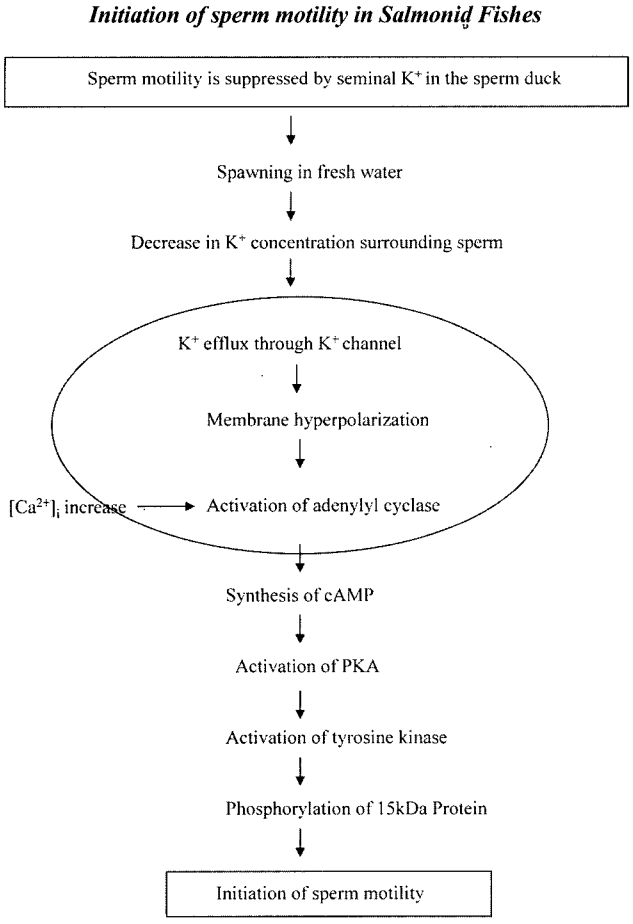

Fig. 1. Transmembrane cell signaling for the initiation of sperm motility in salmonid fishes.
The experimental conditions for demonstrating the role of K^+ in the initiation of sperm motility parallel the natural condition at spawning in K^+-deficient fresh water. $[Ca^{2+}]_i$: intracellular Ca^{2+} concentration.

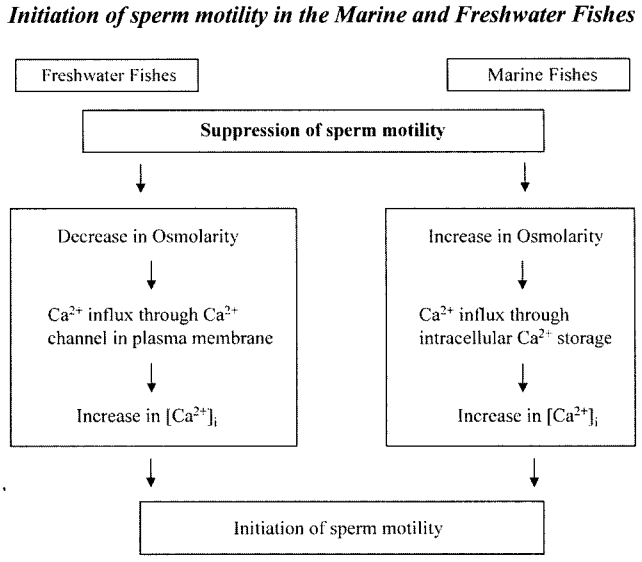

Fig. 2. Transmembrane cell signaling for the initiation of sperm motility in the marine and freshwater fishes.
The experimental conditions demonstrating the role of osmolarity in the initiation of sperm motility parallel the natural spawning conditions in hypotonic freshwater or hypertonic sea water. Generally, the duration of sperm motility in teleosts is relatively short, and mature males approach females and release spermatozoa immediately after oviposition. $[Ca^{2+}]_i$: intracellular Ca^{2+} concentration.

plasma membrane. In salmonid fishes, calmodulin causes the increase in K^+ permeability of the plasma membrane, resulting in hyperpolarization of the plasma membrane to induce cAMP synthesis.

The transmembrane cell signaling underlying the initiation of sperm motility in aquatic species is summarized in Figs. 1 and 2. In salmonid fishes, sperm motility is suppressed by high seminal K^+, ranged 40–80 mM. A decrease in K^+ concentration surrounding spawned sperm in fresh water causes K^+ efflux, through the K^+ channel, resulting in hyperpolarization of the plasma membrane of the sperm flagellum. The change of membrane potential could directly activate adenylyl cyclase, which increases intracellular cAMP concentration. Increased intracellular Ca^{2+} may also activate the enzyme in cooperation with membrane hyperpolarization. Following this process, cAMP activates PKA, resulting in activation of tyrosine kinase and phosphorylation of the 15 kDa protein, which triggers a final step leading to the initiation of sperm motility (Fig. 1). In the marine and freshwater fishes, motility of sperm is suppressed by seminal osmolality of about 300 mOsmol/kg. The released sperm reach the spawned oocyte within a short period. During the approach of sperm to the oocyte, shrinkage or swelling of sperm cells may be caused by the changes in external osmolarities, and the events may cause the increase in $[Ca^{2+}]_i$ by Ca^{2+} influx through the Ca^{2+} channel or Ca^{2+} efflux from the intracellular Ca^{2+} storage. Increases in $[Ca^{2+}]_i$ of sperm cells have critical roles for the initiation of sperm motility in the marine and freshwater fishes (Fig. 2). Compared with the salmonid fishes, cAMP is not required for the initiation of sperm motility in freshwater and marine teleosts.

In this paper, the physiological mechanisms and transmembrane cell signaling of sperm motility in aquatic species have been suggested. Further research on the exact role of calmodulin in transmembrane signal transduction in the initiation of sperm motility is needed in the future.

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