

## Mechanisms for the Initiation of Sperm Motility

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**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<sup>2+</sup> in vertebrates and invertebrates. Mammalian sperm requires Ca<sup>2+</sup> and cyclic AMP for the activation of sperm motility. Cell signaling for the initiation and activation of sperm motility has been well studied in the ascidians, *Ciona intestinalis* and *C. savignyi* and salmonid fishes. In *Ciona*, whose cell signaling for activation of sperm motility has been established, the sperm-activating and -attracting factor released from unfertilized egg requires extracellular Ca<sup>2+</sup> for activating sperm motility and eliciting chemotactic behavior of the activated sperm toward the egg. On the other hand, the cyclic AMP-dependent phosphorylation of protein is essential for the initiation of sperm motility in salmonid fishes. A decrease in the environmental K<sup>+</sup> concentration surrounding the spawned sperm causes a K<sup>+</sup> efflux and Ca<sup>2+</sup> influx through the specific K<sup>+</sup> channel and dihydropyridine-sensitive L-/T- type Ca<sup>2+</sup> channel, respectively, thereby leading to the membrane hyperpolarization and Ca<sup>2+</sup> influx. The membrane hyperpolarization synthesizes cyclic AMP, which triggers the further process of cell signaling, i.e., cyclic AMP-dependent protein phosphorylation, to initiate sperm motility in salmonid fishes.

**Key words** : Sperm, Motility, Cell signaling.

**요약** : 정자의 운동개시는 수정시에 정자와 난자가 만나기 위한 전제조건이다. 동물의 정자는 cAMP와 Ca<sup>2+</sup>의 조절기구에 의해서 정자의 운동개시가 일어난다. 정자운동 활성화 및 개시를 위한 세포 신호전달기구는 멍게류와 연어과 어류에서 많은 연구가 이루어져 왔다. 멍게류의 경우, 난에서 분비되는 정자 활성화 및 유인물질(sperm-activating and -attracting factor)은 정자 활성화 및 난으로의 유인을 위하여 외부의 Ca<sup>2+</sup>를 요구한다. 한편 연어과 어류의 정자에서는 cyclic AMP 의존형의 단백질 인산화가 정자 운동개시 기구에 관여한다. 방정된 정자 주위의 K<sup>+</sup> 농도의 감소는 특정한 K<sup>+</sup> channel 및 dihydropyridine 감수성의 L-/T- type Ca<sup>2+</sup> channel을 통한 K<sup>+</sup> 유출과 Ca<sup>2+</sup> 유입에 의해 세포막의 과분극과 세포내 Ca<sup>2+</sup> 이온의 농도증가를 가져온다. 세포막의 과분극에 의해서 합성된 cyclic AMP는 정자 운동개시의 주요기구인 cyclic AMP 의존형의 단백질 인산화를 유도한다.

### INTRODUCTION

The state of spermatozoan motility can be generally divided into four steps during its life history. The first is in the time of spermatogenesis in which spermatogonia proliferate, grow and metamorphose into morphologically specialized cells, spermato-

zoa. 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. As the third step, the initiation of sperm motility occurs after spermatozoa are spawned or ejaculated. The fourth step involves motility activation or chemo-

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tactic 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, we will review the mechanisms underlying the initiation of sperm motility in several animal species.

### FACTORS INITIATING SPERM MOTILITY IN INTERNAL FERTILIZATION SPECIES

Studies on mechanisms of the initiation and activation of sperm motility at spawning is very useful to understand not only the mechanisms for flagellar movement but also the intracellular signal transduction systems in general. Processes of the initiation and activation of sperm motility are different according to the types of fertilization, external fertilization or internal fertilization. In external fertilization species, the spermatozoa obtain motility just 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, the transmembrane cell signaling for the initiation of sperm motility is controlled by the changes in environmental ionic conditions at spawning of sperm from male reproductive tract to spawning ground, which is fresh water, in the external fertilization species.

In the sperm of mammals, internal fertilization species,  $\text{HCO}_3^-$  and  $\text{Ca}^{2+}$  are well known as the factors for the initiation and activation of sperm motility. It has been reported that external  $\text{Ca}^{2+}$  is required for the activation of motility in bovine, hamster and rat sperm (Morton et al., 1974; 1978; Babcock et al., 1978; 1979). However, the sperm of mouse, rabbit and human are motile upon release from the epididymis, even in the absence of exogenous  $\text{Ca}^{2+}$  (Storey, 1975; Morton et al., 1978; Heffner and Storey, 1981). On the other hand, cyclic nucleotides, particularly cyclic AMP (cAMP), may be the universal factor for the activation of mammalian sperm motility (Morton et al., 1974; Lindermann, 1978).  $\text{HCO}_3^-$  stimulates a synthesis of cAMP from

ATP via activation of adenylyl cyclase, resulting in the activation of porcine (Okamura et al., 1985; Tajima et al., 1987) and hamster sperm motility (Si and Okuno, 1993; Si and Okuno, 1995a). Tash and his colleagues (Tash and Means, 1983; Tash et al., 1984) 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 kDa and 65 kDa proteins regulates the velocity of microtubule sliding in the sperm of hamster (Si and Okuno, 1999a) and mouse (Si and Okuno, 1995a; b), respectively. In these species, 80 kDa tyrosine phosphorylated protein is considered as a hyperactivated-motility producing protein (Si and Okuno, 1999b). 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 demembrated 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 (Ashizawa et al., 1994a; b; 1995).

### FACTORS INITIATING SPERM MOTILITY IN EXTERNAL FERTILIZATION SPECIES

In the sea urchin, a typical external fertilization species, Gray (1928) first described 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 an increased free space for movement. Rothchild (1948) extended the hypothesis to include the effects of gas tension on the initiation of sperm motility. In his hypothesis, changes in environment surrounding the sperm from  $\text{CO}_2$ -rich anaerobic testis to aerobic  $\text{O}_2$ -rich seawater cause the increase in respiration of sperm at mid-piece mitochondria, resulting in supplement of the energy for sperm motility. Johnson et al. (1983) then demonstrated that  $\text{CO}_2$  suppresses sperm metabolism in the seminal plasma. Upon spawning, decrease in  $\text{CO}_2$  of sperm environment induces  $\beta$ -oxidation of lipids composed of 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 became recognized as the transmembrane cell signal for the initiation of sperm motility. Nishioka and Cross(1978) demonstrated that the efflux of  $H^+$  and influx of  $Na^+$  across  $Na^+/H^+$  antiporter cause the initiation of sperm motility. Tombes and Shapiro(1985) further demonstrated that the intracellular alkalization activates dynein ATPase to initiate sperm motility. Resulting consumption of ATP causes the beginning of transport of high energy source for sperm motility(Tombes and Shapiro, 1985).

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, sea urchin and annelids(Lillie, 1919). In the sea urchin, arbia, a decapeptide named speract was purified from egg-jelly. Then the cell signaling, which includes cyclic nucleotides, cyclic nucleotide-gated  $K^+$  channel, hyperpolarization of the plasma membrane(Babcock et al., 1992; Cook and Babcock, 1993a; b; Galindo et al., 2000), alkalization of intracellular pH and increase in intracellular  $Ca^{2+}$  level (Gonzalez-Martinez et al., 1992) was proposed as some factors for initiation of sperm motility. Furthermore, it was suggested that the hyperpolarization activates adenylyl cyclase, and cooperation of the enzyme and increased  $Ca^{2+}$  cause synthesis of cAMP(Beltran et al., 1996; Cook and Babcock, 1993b). The principal intracellular target for cAMP in the cells is the PKA which phosphorylates proteins and regulates cell functions. Ishiguro et al.(1982) have shown that cAMP is prerequisite for the activation of demembrated sea urchin sperm and identified the protein components with PKA activity. In addition to the PKA, another component was proposed to be required for the cAMP-dependent reactivation of the demembrated sperm.

### **AN EGG-DERIVED FACTORS TO INDUCE BOTH ACTIVATION AND CHEMOTAXIS IN ASCIDIAN SPERM**

The cell signaling mechanisms for the activation of motility and attraction of sperm have been investigated in the ascidians, *Ciona intestinalis* and *C. savignyi*. The binding of sperm-activating and -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 the

membrane hyperpolarization. This hyperpolarization of the membrane potential activates the adenylyl cyclase to elevate the cAMP level in the sperm cytoplasm(Izumi et al., 1999). Cyclic AMP activates PKA and phosphorylates 26 kDa protein and the dynein light chain(Nomura et al., 2000). 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 of the rainbow trout(Yoshida and Nomura, 1972). A factor responsible for the activation and agglutination of spermatozoa is localized around the micropylar entrance of the egg membrane in bitteling and fat minnow(Suzuki, 1959). Amanze and Iyenger(1990) reported that the sperm of the rosy barb exhibited searching activity for the micropylar entrance of the egg. These results suggest that there is a general mechanism in teleosts in which the spermatozoa could be fully activated by the sperm-activating substance released from the egg to help spermatozoa reach the plasma membrane of the egg at fertilization. The pacific herring is unique among marine teleosts from a viewpoint of the 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 that exist around the egg cause the the initiation and activation of sperm motility(Morisawa et al., 1992). The substance was purified and named as the herring sperm activating proteins, HSAPs, by Oda et al.(1995). Identification of molecular structure of the HSAPs by cDNA cloning revealed that the HSAPs are homologous to the trypsin inhibitors(Oda et al., 1998). Furthermore, Yoshida(1998) found several proteins with binding capacity to HSAPs. Among them, prolyl endopeptidase that exists in the sperm surface was proposed as a receptor of the HSAPs. On the other hand, Yanagimachi(1957) found another sperm activating factor that is tightly bound to the egg surface, and is not easily diffusible into the seawater. Pillai et al.(1993) purified this sperm-activating protein sizes at 105 kDa and named sperm motility initiation factor(SMIF). The factor is localized around the micropyle area of the unfertilized mature eggs (Yanagimachi et al., 1992; Griffin et al., 1996). These suggested that sperm activated by 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  $\text{Ca}^{2+}$ .

### 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 (Morisawa and Suzuki, 1980). The role of the environmental factors for the initiation of sperm motility in teleost fishes has been well characterized by Morisawa and his colleagues. They found the physiological factors that can act as an on-off switch of sperm motility in these groups of teleost fish (Morisawa and Suzuki, 1980; Morisawa et al., 1983). In marine and freshwater teleosts, spermatozoa are activated when they are exposed to changes in osmolality surrounding the sperm at spawning (Morisawa and Suzuki, 1980; Morisawa et al., 1983). Sperm of puffer fish (Morisawa and Suzuki, 1980), sea bass (Billard, 1978), black sea bream (Chang et al., 1995), marbled sole (Kho et al., 1997), gray mullet (Chao et al., 1975; Chang et al., 1999), goby (Weisel, 1948), halibut (Billard et al., 1993) and Atlantic croaker (Detweiler and Thomas, 1998) initiate motility when they are suspended into hyperosmotic water. On the other hand, sperm of freshwater cyprinid fish (Morisawa and Suzuki, 1980), goldfish (Morisawa and Suzuki, 1980), common carp (Billard, 1978; Krasznai et al., 2000), zebra fish (Takai and Morisawa, 1995) and pejerrey (Strüssmann et al., 1994), initiate motility when they are suspended into hypoosmotic water. Takai and Morisawa (1995) demonstrated that the changes in intracellular  $\text{K}^+$  ion concentration directly regulates sperm motility via change in external osmolality in marine and freshwater teleosts. Extracellular divalent cation,  $\text{Ca}^{2+}$ , is involved in the signaling cascade for the initiation of sperm motility in teleosts of not only osmolality-dependent but also  $\text{K}^+$  decrease-dependent species. In the common carp, sperm motility is initiated depending on the extracellular  $\text{Ca}^{2+}$  and thus is initiated by  $\text{Ca}^{2+}$  channel blockers of both 1,4-dihydropyridine- and peptide-types (Krasznai et al., 2000).

### $\text{K}^+$ -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 this species, high concentration of  $\text{K}^+$  in the seminal plasma suppresses the sperm motility in the male reproductive tract. Decrease in  $\text{K}^+$  surrounding the sperm synthesizes cAMP (Morisawa and Ishida, 1987). The cAMP (Morisawa and Okuno, 1982) activates cAMP-dependent protein kinase (PKA). The resultant phosphorylation of the outer arm dynein light chain (Inaba et al., 1999) and tyrosine kinase to phosphorylate 15 kDa protein (Morisawa and Hayashi, 1985) triggers the final step of the initiation of flagellar movement. Despite such 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 (Tanimoto and Morisawa, 1988; Tanimoto et al., 1994; Gatti et al., 1990; Boitano and Omoto, 1991), efflux of  $\text{K}^+$  and influx of  $\text{Ca}^{2+}$  (Cosson et al., 1989; Tanimoto et al., 1994) have been suggested so far. However, the transmembrane cell signaling cascade i.e. the relationship among  $\text{K}^+$  channels,  $\text{Ca}^{2+}$  channels, membrane potentials and cAMP synthesis is obscure.

Membrane hyperpolarization through a kind of voltage dependent  $\text{K}^+$  channels causes cAMP synthesis to lead to the initiation of sperm motility in salmonid fishes such as rainbow trout, steelhead and so on (Kho et al., 2001a; 2003).  $\text{Ca}^{2+}$  influx through a kind of dihydropyridine sensitive L-/T-type  $\text{Ca}^{2+}$  channel may also participate in the membrane hyperpolarization and synthesis of cAMP, which is the intracellular trigger for the initiation of sperm motility (Kho, 2001b).

Calmodulin, a calcium-binding protein, has been discovered as an activator of cyclic nucleotide phosphodiesterase (Cheung, 1970; Kakiuchi and Yamazaki, 1970). This protein acts as an intracellular receptor for  $\text{Ca}^{2+}$  and plays a wide variety of roles in cell signaling in eukaryotic cells (reviewed in Means et al., 1982), i.e. cyclic nucleotide metabolism, glycogen metabolism (Epel et al., 1981), cell cycle (Chafouleas et al., 1979) and so on. In regard to cell motility,  $\text{Ca}^{2+}$ /calmodulin is required for the optimal activity of myosin light chain kinase to phosphorylate the 20 kDa light chain of myosin (Pires and Perry, 1977).  $\text{Ca}^{2+}$ /calmodulin also plays a significant role for polymerization

and depolarization of tubulin in the formation and degradation of mitotic spindle in the process of mitotic cell division (Mazia et al., 1972). Calmodulin may play a role in the regulations of flagellar motility as a  $\text{Ca}^{2+}$  sensor to change the flagellar bending pattern from symmetric wave to asymmetric wave and vice versa according to the intracellular  $\text{Ca}^{2+}$  concentration in sea urchins (Brokaw and Nagayama, 1985; Brokaw, 1991). In sea urchins, adenylyl cyclase has been purified as a protein with an affinity to calmodulin (Bookbinder et al., 1990). It has been also reported that calmodulin activates ATPase activity of dynein isolated from the cilia of *Tetrahymena pyriformis* (Blum et al., 1980). In the ascidians, it has been reported that SAAF from the egg causes  $\text{Ca}^{2+}$  entry, and the  $\text{Ca}^{2+}$  activates calmodulin and calmodulin kinase II to hyperpolarize the plasma membrane.

In salmonid fishes, calmodulin causes the increase in  $\text{K}^+$  permeability of the plasma membrane, resulting in hyperpolarization of the plasma membrane to lead to a cAMP synthesis. However, the exact role of calmodulin on the transmembrane cell signaling for the initiation of sperm motility still remained to be solved.

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