



---

## **Molecular Architecture of the Sperm Flagella: Molecules for Motility and Signaling**

Author: Inaba, Kazuo

Source: Zoological Science, 20(9) : 1043-1056

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.20.1043>

## [REVIEW]

# Molecular Architecture of the Sperm Flagella: Molecules for Motility and Signaling

Kazuo Inaba\*

*Asamushi Marine Biological Station, Graduate School of Science, Tohoku University,  
Sakamoto 9, Asamushi, Aomori, Aomori 039-3501, Japan*

**ABSTRACT**—Sperm motility is generated by a highly organized, microtubule-based structure, called the axoneme, which is constructed from approximately 250 proteins. Recent studies have revealed the molecular structures and functions of a number of axonemal components, including the motor molecules, the dyneins, and regulatory substructures, such as radial spoke, central pair, and other accessory structures. The force for flagellar movement is exerted by the sliding of outer-doublet microtubules driven by the molecular motors, the dyneins. Dynein activity is regulated by the radial spoke/central pair apparatus through protein phosphorylation, resulting in flagellar bend propagation. Prior to fertilization, sperm exhibit dramatic motility changes, such as initiation and activation of motility and chemotaxis toward the egg. These changes are triggered by changes in the extracellular ionic environment and substances released from the female reproductive tract or egg. After reception of these extracellular signals by specific ion channels or receptors in the sperm cells, intracellular signals are switched on through tyrosine protein phosphorylation,  $\text{Ca}^{2+}$ , and cyclic nucleotide-dependent pathways. All these signaling molecules are closely arranged in each sperm flagellum, leading to efficient activation of motility.

**Key words:** sperm, motility, flagella, axoneme, dynein

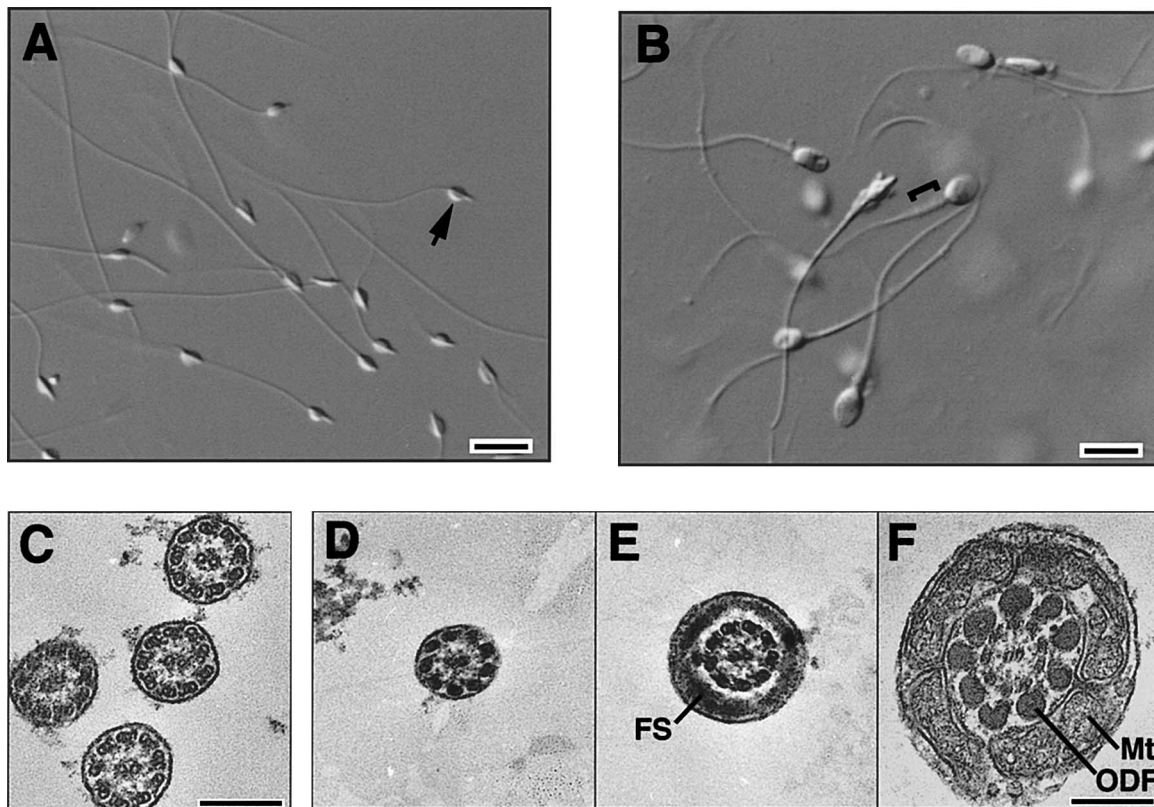
## INTRODUCTION

As the tails of sperm, flagella comprise the motile apparatus necessary for the movement and penetration of sperm into the egg at fertilization (Fig. 1A, B). They show oscillatory movements at high speed. The motility is generated by the internal cytoskeletal structure called the axoneme, which is a highly organized microtubule-based structure that has been well conserved through evolution (Fig. 1C). The axoneme also serves as the motility or sensory apparatus of cilia on the trachea, oviduct, and sensory organs. Mammalian sperm flagella are divided into two parts, the midpiece and the principal piece, and accessory structures are present between the axonemes and the plasma membrane (Fig. 1B). In the midpiece of the flagellum, the axonemes are surrounded by outer dense fibers (ODF) and mitochondria, while a fibrous sheath (FS) surrounds the axoneme in the principal piece (Fig. 1D–F) (for review, see Baccetti and Afzelius, 1976).

Immediately after spermiogenesis, sperm cells show no or little motility. Prior to fertilization, however, sperm motility changes dramatically. For example, sperm from animals with external fertilization show activation of motility by changes in the extracellular ionic environment or by substances released from the egg. Sperm from animals with internal fertilization show activation by factors in the female reproductive tract or substances from the egg. All of these processes are triggered by reception of the respective stimuli. The motility machinery, the axoneme, is finally activated as the end-stage of the intracellular signaling pathway (Morisawa, 1994; Darszon *et al.*, 2001). The molecules involved in this signaling pathway appear to be highly organized, as are the axonemes, to facilitate such a prompt response.

The structure and function of axonemes have been widely studied in *Chlamydomonas* using several mutants with motility defects (reviewed in Mitchell, 2000). Recent studies have explored the molecular architecture of the axoneme in detail. In addition, the signaling mechanism for modulation of sperm motility has been clarified by the recent identification of several molecules, including receptors,

\* Corresponding author: Tel. +81-17-752-3394;  
FAX. +81-17-752-2765.  
E-mail: inaba@biology.tohoku.ac.jp



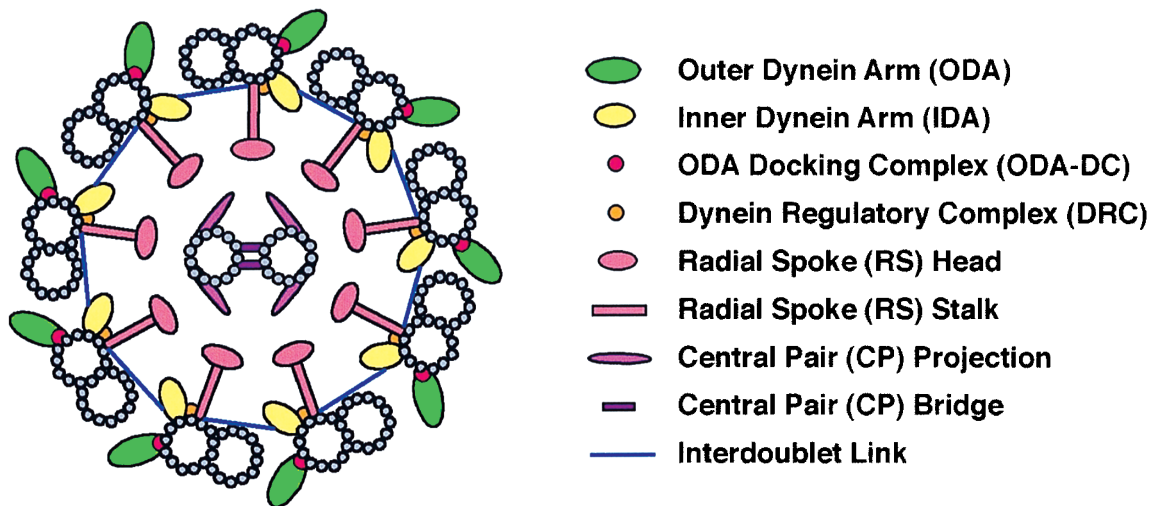
**Fig. 1.** Morphology of ascidian and mammalian sperm. **A**, DIC image of sperm from the ascidian *Ciona intestinalis*. The arrow shows a mitochondrion attached to the side of a nucleus. Bar, 10  $\mu$ m. **B**, DIC image of human sperm. The region of the midpiece is indicated. The rest of the flagellum is the principal piece, except for the small portion at the tip called the endpiece. Bar, 10  $\mu$ m. **C**, Electron microscopic image of the cross-section of *Ciona* flagellum. The axonemal 9+2 structure is surrounded by plasma membrane. Bar, 200 nm. **D–F**, Electron microscopic images of several portions of human sperm flagella. **D**, endpiece; **E**, principal piece; **F**, midpiece. The endpiece has no accessory structure between the axoneme and the plasma membrane. A fibrous sheath (FS) is present between them in the principal piece. In the midpiece, the axoneme is surrounded by nine rows of outer dense fiber (ODF), which are further wrapped by mitochondria (Mt). The scale bar in D–F indicates 200 nm.

channels, and signal molecules, involved in the activation of motility. Functional genomics and proteomics are expected to provide new ways to carry out extensive characterization of the components of flagella. In this review, molecules constructing sperm flagella and essential for the regulation of sperm motility are described. The detailed structure and function of each protein molecule have been described in previous review articles (Gibbons, 1981; Kamiya, 2002; Morisawa, 1994; Ho and Suarez, 2001; Darszon *et al.*, 2001; Garbers, 1989).

### I. Axonemes: The motility apparatus of sperm flagella

The 9 + 2 structure and molecular composition of the axoneme are well conserved among eukaryotic cilia and flagella from protozoans to human (Fig. 2). The doublet microtubules, numbered from 1 to 9, are composed of a complete A-tubule and an incomplete B-tubule. The central microtubules are named C1 and C2. Several structures are bound to these microtubules and comprise a highly organized protein network (Fig. 2). Axonemes are composed of approximately 250 proteins. Extensive studies of the molecular composition of axonemes have been carried out with the

green alga *Chlamydomonas*. However, more than half of the protein components remain to be characterized. Knowledge of *Chlamydomonas* axonemes can be applied to sperm axonemes, but there are some differences in the composition and molecular structure between *Chlamydomonas* and metazoan sperm. Recent molecular studies to identify axonemal components revealed that the axoneme is a sophisticated structure with a cytoskeleton, protein motors, molecular chaperones,  $\text{Ca}^{2+}$ -binding proteins and protein kinases / phosphatases. Some axonemal proteins possess motifs that are potentially essential for protein-protein interactions, facilitating the assembly of such a highly organized structure. The axonemes are generated from the basal body where a set of transient structures are connected to the axoneme. Axonemal components are integrated through a microtubule-dependent transport system, called intraflagellar transport (IFT). The basal body and its associated structure, the composition of IFT particles, and the mechanism of IFT have been reviewed elsewhere (Marshall and Rosenbaum, 2000; Rosenbaum and Witman, 2002).



**Fig. 2.** Substructures in the axoneme. Axonemes are constructed from nine doublet microtubules and two singlet microtubules as shown by gray rings. Each doublet microtubule is a unit for sliding, with dynein arms, the dynein docking complex, radial spokes, and the dynein regulatory complex. Nine doublet microtubules are connected with each other by interdoublet links. The singlet microtubules at the center are joined by the central pair bridge. Each microtubule possesses two central pair projections. The molecular composition of each substructure is detailed in the text.

### Dynein arms

Dyneins are microtubule-dependent force-generating ATPases. The dyneins involved in the motility of eukaryotic cilia and flagella are classified as axonemal dyneins. Dyneins are observed as a pair of projecting “arms” on doublet microtubules designated the outer and inner arms. The structure of outer arm dynein has been studied extensively. In metazoan sperm, it is comprised of two heavy chains (~500 kDa) (three in *Chlamydomonas*), three to five intermediate chains (120–60 kDa) (two in *Chlamydomonas*), and six (eight in *Chlamydomonas*) light chains (30–8 kDa).

**Heavy chains:** Dynein heavy chains are huge proteins with a molecular mass of ~500 kDa. The outer arm dynein contains two heavy chains,  $\alpha$  and  $\beta$ , which are related phylogenically to  $\gamma$  and  $\alpha/\beta$  heavy chains of *Chlamydomonas* outer arm dynein, respectively. Each heavy chain appears to play a distinct function in microtubule-sliding. They contain four P-loop ATP-binding motifs (Ogawa, 1991; Gibbons *et al.*, 1991). Recent studies have shown that dynein heavy chains are members of the AAA superfamily and have six AAA domains (Mocz and Gibbons, 2001; King, 2000b). Each AAA motif is considered to form a globular subdomain. The coiled-coil domain present between the fourth and fifth AAA motif forms a small stem that is essential for ATP-sensitive binding to the adjacent B-tubule (King 2000a; Gee *et al.*, 1997). The interzone between each AAA domain appears to be important for the conformational change during mechanochemical cycles (Inaba and Mohri, 1989; Inaba, 2000), which presumably exerts the power stroke (Burgess *et al.*, 2003).

**Intermediate chains:** *Chlamydomonas* outer arm dynein contains two intermediate chains, IC78 and IC69, of which

clear homologs are present in sperm flagella (Ogawa *et al.*, 1995). Both possess WD-repeats, which are involved in protein-protein interaction and possibly play a key role in assembly and binding of dynein on the A-tubule. Sperm flagella contain a unique intermediate chain with thioredoxin and nucleoside diphosphate kinase (TNDK-IC) motifs (Ogawa *et al.*, 1996; Padma *et al.*, 2001). In sea urchin sperm flagella, this intermediate chain shows a high molecular mass (~120 kDa), but the size of TNDK-IC has become smaller during evolution (Padma *et al.*, 2001). In *Ciona*, salmonid fish and mollusca, outer arm dynein contains two or three other intermediate chains (Ogawa *et al.*, 1996; Padma *et al.*, 2001), but these remain uncharacterized.

**Light chains:** There are six or eight distinct proteins identified as outer arm dynein light chains in metazoan sperm flagella and *Chlamydomonas* flagella, respectively (Inaba *et al.*, 1998, 1999; King, 2000). Two of these molecules show homology to t-complex testis-expressed proteins (Tctex1 and Tctex2), which are involved in transmission ratio distortion in mouse (Olds-Clarke, 1997). The Tctex2-related dynein light chain is phosphorylated at activation of sperm motility in a cAMP-dependent manner and may play a key role in the activation of outer arm dynein (Inaba *et al.*, 1999). The other molecules include a leucine-rich repeat (LRR) protein and two isoforms homologous to highly conserved *Chlamydomonas* 8-kDa light chains. Tctex1 light chain is not present in the outer arm dynein of *Chlamydomonas*. One light chain (LC5) remains to be identified. A small protein with homology to *Drosophila* roadblock has been identified in *Chlamydomonas* (King, 2002) and it could be a possible candidate for sperm LC5. Although *Chlamydomonas* outer arm dynein contains  $\text{Ca}^{2+}$ -binding light chain but no  $\text{Ca}^{2+}$ -binding proteins have been found in metazoan outer arm

dynein. Calmodulin (CaM) was found to be associated with outer arm dynein in sea urchin and mammalian sperm flagella (Tash *et al.*, 1988). A 25-kDa protein with sequence similarity with calcineurin B subunit has recently been identified in association with *Ciona* outer arm dynein (Padma and Inaba, manuscript in preparation).

**Outer arm docking complex:** The factor for assembly of outer arm dynein to the doublet microtubules at regular intervals (24 nm) was first identified in *Chlamydomonas* (Takada and Kamiya, 1994) and was designated outer dynein arm docking complex (ODA-DC). This molecule sediments at 7 S on sucrose density gradient centrifugation and is composed of three polypeptides. Both *Chlamydomonas* DC1 and DC2 are coiled coil proteins that may be involved in scaling on microtubules for binding of outer arm dynein at regular intervals (Koutoulis *et al.*, 1997; Takada *et al.*, 2002). DC3 has four EF-hand motifs that possibly bind  $\text{Ca}^{2+}$  and have some roles in the regulation of outer arm dynein (Casey *et al.*, 2003). We have recently identified a DC2 homolog (CiAx p66.0) in *Ciona* sperm flagella, but part of this protein was isolated bound to outer arm dynein (Ushimaru *et al.*, manuscript in preparation; Accession number, AB083180). However, proteins with significant similarity to DC1 and DC3 could not be identified in metazoans. Although similar ODA-DC may be present in metazoan sperm, its composition and binding properties to doublet microtubules may be different between *Chlamydomonas* and metazoan sperm flagella.

**Subunits of inner arm dynein:** Inner arm dyneins are more complex than outer arm dyneins, and include multiple molecular species with more heavy chains. Inner arm dyneins in *Chlamydomonas* have been shown to contain seven species (a-f) (Kamiya, 2002). The inner arms of eel sperm axonemes are morphologically divided into three species (Woolley, 1997), similar to those in *Chlamydomonas* (Goodenough and Heuser, 1985). *Chlamydomonas* f inner arm dynein (also called I1) contains intermediate chains IC140, IC138, IC97, the 14-kDa Tctex1 light chain, and an 8-kDa light chain (Kamiya, 2002). IC138 is regulated by phosphorylation / dephosphorylation through a kinase/phosphatase system present in the radial spoke and central pair in response to changes in motility (Habermacher and Sale, 1997; King and Dutcher, 1997). A homolog of IC140 (Ci-IC116) has recently been isolated from sperm flagella of the ascidian *Ciona intestinalis*. Similarly to *Chlamydomonas* IC140 (Yang and Sale, 1998; Perrone *et al.*, 1998), *Ciona* IC116 contains WD repeats, suggesting that it is involved in the assembly or anchoring of inner arm dyneins to the microtubule. However, Ci-IC116 appears to be dephosphorylated at activation of motility, suggesting a regulatory role of this protein (Inaba *et al.*, 2002). *Chlamydomonas* inner arm dyneins contain p28, actin, centrin, and Tctex1 and a few unidentified proteins as light chains (Kamiya, 2002). A sea urchin homolog of *Chlamydomonas* p28 (p33) was

reported to be present in axonemes as a putative inner arm dynein light chain (Gingras *et al.*, 1996), but its detailed localization has not been elucidated. Actin is found in inner arm dyneins in association with a p28 homolog in fish sperm (King *et al.*, 1997), as in the case of *Chlamydomonas* (Piperno *et al.*, 1990; Kato-Minoura *et al.*, 1997). A set of proteins form a complex at the junction between radial spokes and inner arm dynein, called the dynein regulatory complex (DRC) (Piperno *et al.*, 1994; Gardner *et al.*, 1994). Seven polypeptides (29-192 kDa) were proposed as DRC components in *Chlamydomonas* but neither molecular characterization nor the presence of homologs in metazoan sperm have been reported.

**Radial spoke and central pair:** Several lines of evidence support the idea that the central pair determines the plane of flagellar bending by sending signals to radial spokes (Smith and Lefebvre, 1997b; Nakano *et al.*, 2003). It was recently suggested that the C1 microtubule is oriented toward the position of active sliding (Wargo and Smith, 2003). Radial spokes regulate inner arm dynein through protein phosphorylation / dephosphorylation (Porter and Sale, 2000). In fact, among the 22 polypeptides identified to date as radial spoke proteins in *Chlamydomonas* flagella, 97-kDa RSP3 (radial spoke protein 3) has been shown to be located at the base of the radial spoke stalk and possesses an AKAP domain that anchors a cAMP-dependent protein kinase (A-kinase) (Gaillard *et al.*, 2001). RSP3 of *Ciona* axoneme also has an AKAP domain but its entire length is much shorter than that of *Chlamydomonas* RSP3 (Padma *et al.*, 2003). CaM is also a component of radial spokes and may play a role in  $\text{Ca}^{2+}$ -dependent changes in the flagellar waveform (Yang *et al.*, 2000). Radial spokes also contain RSP4/6 in the spoke head. RSP4/6 is also a component of sea urchin sperm radial spokes (Gingras *et al.*, 1998). Recently, a novel leucine-rich repeat (LRR) component of the radial spoke head (LRR37) has been identified (Padma *et al.*, 2003). In view of the possible function of LRR in protein-protein interaction, it might be involved in the interaction with other components of the radial spoke or with components of central pair projection.

Some proteins constituting central pair projections have been identified using *Chlamydomonas* mutants (Mitchell, 2000). Approximately 23 polypeptides (14-360 kDa) comprise the structure associated with central pair microtubules (Adams *et al.*, 1981; Dutcher *et al.*, 1984). Similarly to the radial spokes, the central pair also contains an AKAP (AKAP240) (Gaillard *et al.*, 2001). Genes for PF6 (alanine/proline-rich protein; Rupp *et al.*, 2001), PF16 (armadillo repeat protein; Smith and Lefebvre, 1996), and PF20 (WD repeat protein; Smith and Lefebvre, 1997a) have been cloned and well characterized in *Chlamydomonas*. Orthologs of PF16 (Spag6) and PF20 are associated with each other in mammalian sperm (Sapiro *et al.*, 2002; Zhang *et al.*, 2002). However, there are no orthologs with significant homology to PF6 over the entire length of the sequence

in metazoan sperm. A kinesin-related protein, KLP1, and a 110-kDa protein that is immunologically related to a kinesin, were identified as components of the central apparatus in *Chlamydomonas* (Bernstein *et al.*, 1994; Johnson *et al.*, 1994). The precise function and the presence of the kinesin-related protein in the central pair of sperm flagella are unknown, although subunits of kinesin II appear to be localized at the midpiece of sea urchin sperm and seem to be involved in intraflagellar transport (Henson *et al.*, 1997).

**Other proteins in relation to axonemes:** Seven members of the tubulin superfamily, the  $\alpha$ - to  $\epsilon$ -tubulins, have been identified (Dutcher, 2001). The  $\gamma$ -,  $\delta$ -,  $\epsilon$ -,  $\zeta$ -, and  $\eta$ -tubulins are localized at the basal body and are probably involved in construction of the centriole or formation of the axoneme. In mouse sperm,  $\delta$ -tubulin is localized in several parts of the sperm cells, including the principal piece of the flagellum (Smrzka *et al.*, 2000), suggesting a distinct function of this tubulin in the axonemal architecture. The  $\alpha$ - and  $\beta$ -tubulins undergo posttranslational modification, such as acetylation, palmitoylation, tyrosine phosphorylation, polyglutamylation and polyglycylation. These modifications play roles in microtubule functions, such as microtubule stability and the interaction with associated proteins (Huitorel *et al.*, 1999). Some of these modifications, such as polyglutamylation, apparently participate in axonemal motility (Gagnon *et al.*, 1996).

Flagellar ribbons are Sarkosyl-resistant structures that are localized along the A-tubule and may play a role in the three-dimensional organization of the axoneme (Norrander *et al.*, 1996). Ribbons are composed of three fibrous proteins called tektins (53, 51, and 47 kDa), along with 83-kDa, 77-kDa, and several lower molecular mass proteins in sea urchin sperm flagella (Hinchcliffe and Linck, 1998). The ribbons presumably connect with interdoubt links and are involved in the architecture of the ninefold axonemal remnant after solubilization of the outer doublet microtubules (Stephens *et al.*, 1989). A cognate of the heat shock protein HSP70 also seems to be associated with this remnant (Stephens and Lemieux, 1999), but the function of this cognate molecule with regard to the role of HSP70 as a molecular chaperone remains to be determined. Recently, HSP40 was identified as an axonemal component of the *Ciona* sperm axoneme (Padma *et al.*, 2003). HSP40 itself functions as a molecular chaperone but is known to also act as a co-chaperone of HSP70. Preliminary experiments showed that HSP40, like HSP70, is resistant to extraction by low ionic strength solution, suggesting the possibility that the HSP70 cognate and HSP40 form a complex and function in the architecture of doublet microtubules.

Interdoubt links, or "nexin" linkages, are the structures that link the outer doublet microtubules. From the results obtained by observation of the outer arm-less axonemes from eel sperm, however, it has been proposed that the interdoubt link is the major part of the DRC (Wooley, 1997). Using selective extraction, the interdoubt links were shown to be comprised of certain polypeptides (Stephens,

1970). Recently, a strong candidate as a component of interdoubt links, Rib72, with DM10 repeats and EF-hands, was cloned from *Chlamydomonas* as a ribbon component (Ikeda *et al.*, 2003). This protein has since been shown to be the p72 regulatory subunit of  $\text{Ca}^{2+}$ -regulated nucleoside-diphosphate kinase (Patel-King *et al.*, 2002). A homolog of this protein is present in *Ciona* and human, suggesting that it is also a component of sperm flagella.

**Protein kinases / phosphatases and  $\text{Ca}^{2+}$ -binding proteins associated with axonemes:** Protein phosphorylation plays essential roles in the regulation of axonemal movement (see below). The catalytic subunit of cAMP-dependent protein kinase in sperm flagella has a unique testis-specific structure. In salmonid fish (Itoh *et al.*, 2003) and mammals (San Agustin *et al.*, 1998; Agustin *et al.*, 2000), the testis-specific PKA catalytic subunit has a lower molecular weight with a shorter N-terminus sequence, which may be involved in anchoring to the microtubules. PKA is located in the vicinity of outer arm dynein, which is consistent with the prompt phosphorylation of Tctex2-related dynein light chain observed on activation of motility (Inaba *et al.*, 1999; Itoh *et al.*, 2003). The t-complex responder has been shown to be a protein kinase (Smok) with similarities to members of the MARK Ser/Thr protein kinase family (Herrmann *et al.*, 1999). However the substrate proteins of Smok have not been identified in sperm.

In salmonid fish sperm, proteasomes are also located near the outer arm dynein, and these have been suggested to regulate PKA activity (Inaba *et al.*, 1993, 1998). Interestingly, a proteasome-containing structure extends to the plasma membrane, suggesting a linkage from the plasma membrane to the outer arm dynein (Inaba *et al.*, 1998). *Chlamydomonas* axonemes contain casein kinase I associated with doublet microtubules (Yang and Sale, 2000). Although casein kinase I is present in mammalian sperm, it is not clear whether it is associated with sperm flagellar axonemes (Chaudhry *et al.*, 1991).

Tyrosine phosphorylation of sperm proteins also plays crucial roles in the regulation of flagellar motility in sperm (Hayashi *et al.*, 1987; Visconti *et al.*, 1995; Dey and Brokaw, 1991; Si and Okuno, 1999). Several proteins have been reported to be phosphorylated, possibly in relation to motility activation of sperm, including hexokinase (Carrera *et al.*, 1996), AKAP82 (Carrera *et al.*, 1996), a 15-kDa protein in the base of the flagellum (Jin *et al.*, 1994), glycogen synthase kinase (Vijayaraghavan *et al.*, 2000), and many uncharacterized proteins. Most tyrosine phosphorylation is regulated by cAMP-dependent protein kinase (Hayashi *et al.*, 1987; Visconti *et al.*, 1995). Although tyrosine phosphorylation of these proteins seems to be associated with sperm motility, no evidence has been reported for the presence of tyrosine-phosphorylated axonemal components.

Both type 1 and 2A protein phosphatases are associated with flagellar axonemes in *Chlamydomonas* (Yang *et al.*, 2000). However, both were extracted from sperm fla-



gella in salmonid fish sperm using Triton X-100 (Inaba, 2002), although type I protein phosphatase appears to be associated with the axonemes and regulate sperm motility in fowl (Ashizawa *et al.*, 1998).  $\text{Ca}^{2+}$ -dependent type 2B protein phosphatase was reported to be bound to the axoneme in sea urchin and mammalian sperm and may be involved in the regulation of dynein phosphorylation (Tash *et al.*, 1988).

Radial spokes and central pairs contain AKAP as a component as described above. In addition to radial spokes, several AKAPs are known to be present in mammalian sperm, such as AKAP82, AKAP110, and AKAP220 (Vijayaraghavan *et al.*, 1999; Reinton *et al.*, 2000). AKAP82 is a major component of the fibrous sheath of mammalian sperm flagella (Carrera *et al.*, 1994). AKAP28 has recently been identified as a component of airway ciliary axonemes in human (Kultgen *et al.*, 2002). This protein is expressed at high levels in the testis, suggesting that it is also an axonemal component of sperm flagella, although its precise localization remains to be determined.

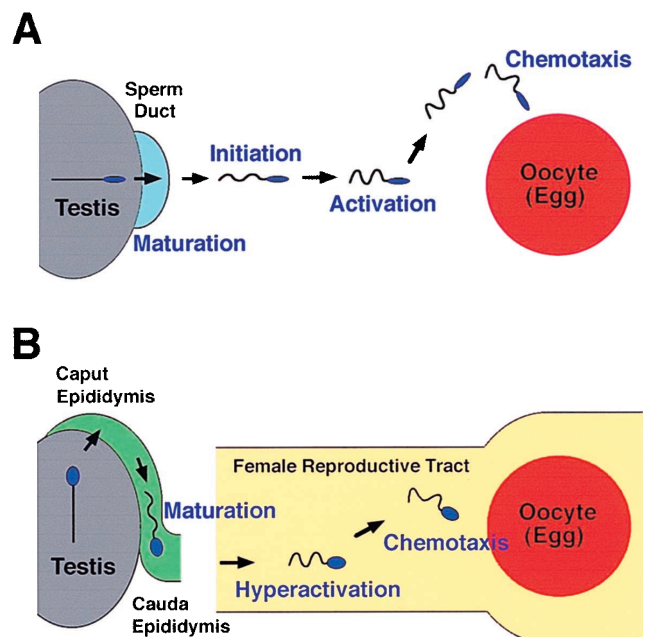
Changes in the direction of sperm movement are caused by modulation of the beating of flagellar waves, a process that involves  $\text{Ca}^{2+}$ -dependent changes in flagellar asymmetry (Brokaw, 1979). The RS/CP system is a target of  $\text{Ca}^{2+}$  (Bannai *et al.*, 2000; Smith, 2002) through calcium-binding proteins, such as CaM, several CaM-binding proteins (Wasco *et al.*, 1989; Ueno *et al.*, 2003), or calsequestrin-like  $\text{Ca}^{2+}$ -binding protein (Berruti and Porzio, 1990). As described above, certain  $\text{Ca}^{2+}$ -binding proteins are components or associated proteins of outer arm dynein, which may also be involved in the asymmetrical axonemal movement.

**Outer dense fiber and fibrous sheath in mammalian sperm:** ODF has been reported to be composed of several cysteine- and proline-rich, intermediate filament-like proteins (Olson and Sammons, 1980; Vera *et al.*, 1984; Oko, 1988). At least 14 polypeptides have been identified as ODF components and are believed to play roles in maintenance of this passive elastic structure (Oko, 1988). Some ODF components and associated proteins have been cloned. ODF1 contains leucine zipper motifs which appear to be responsible for self-interaction and interaction with other ODF components (Shao *et al.*, 1997). FS is composed of at least 18 polypeptides (Oko, 1988) and seems to serve as a scaffold for several enzymes for energy metabolism and as a signaling molecule for sperm motility. FS is known to be comprised of several proteins, including AKAPs (AKAP3, AKAP4, TAKAP80), hexokinase (HK1-S), and Rho-binding protein raphilin and its binding protein roporin (reviewed in Eddy *et al.*, 2003). Interestingly, raphilin is located on the inner surface of FS facing the axoneme (Fujita *et al.*, 2000). This may be related to the tyrosine phosphorylation of FS components, such as HK-1 and AKAP3 (Carrera *et al.*, 1994; Mori *et al.*, 1998) or to FS sliding (Si and Okuno, 1995).

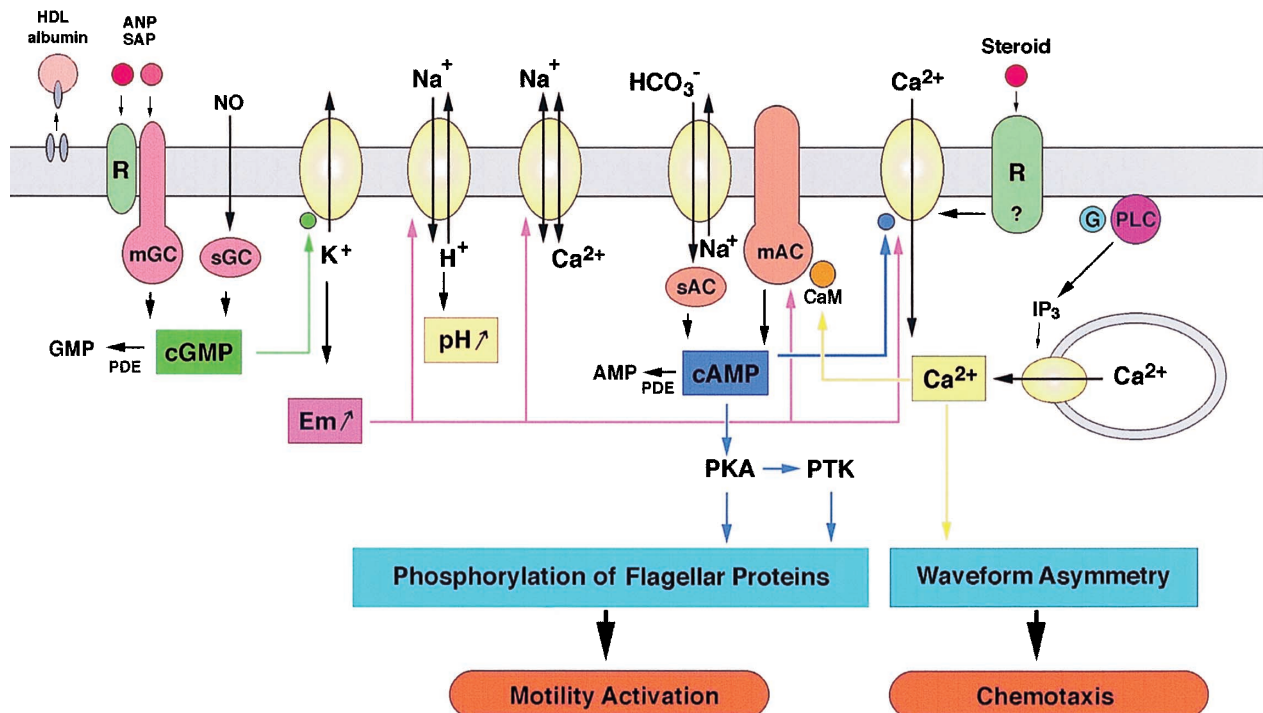
## II. Molecules involved in motility regulation

Sperm motility changes prior to fertilization (Fig. 3). Sperm are immotile in testis even after the completion of spermiogenesis. In animals that undergo external fertilization, sperm initiate motility upon spawning triggered by extracellular ionic changes or substances released from the egg. In fish, sperm appear to undergo maturation in the sperm duct, which is a prerequisite for motility initiation (Morisawa and Morisawa, 1988). Mammalian sperm mature to acquire the ability for motility while passing through the epididymis. Sperm cells ejaculated into the female reproductive tract undergo capacitation. Capacitated sperm show vigorous movements called hyperactivation (Yanagimachi, 1970). In almost all organisms, substances from the egg induce changes in the direction of sperm movement, resulting in chemotaxis toward the egg (Miller, 1985; Cosson, 1990).

Activation of sperm flagellar motility is triggered by reception of ionic changes or substances from the female reproductive tract or the egg. It involves activation of both energy metabolism and the motile apparatus. The former includes many enzymes for ATP production and ATP regeneration (Mohri, 1957; Mita and Yasumasu, 1983; Tombes



**Fig. 3.** Changes in sperm motility prior to fertilization. *A*, Sperm from animals with external fertilization initiate motility at spawning, triggered by extracellular ionic changes or by substances released from the egg. In fish sperm, specific changes or maturation of motility occur while passing through the sperm duct. Factors released from the egg cause sperm to undergo activation and chemotaxis toward the egg. *B*, Sperm from animals with internal fertilization show no motility just after spermiation into the caput epididymis. While passing through the epididymis, they undergo maturation and acquire motility. After ejaculation into the female reproductive tract, their motility changes dramatically. The sperm undergo capacitation and exhibit hyperactivation of motility. A specific substance in the tract shows chemotactic activity toward the sperm.



**Fig. 4.** Transmembrane signaling pathway for the activation of sperm motility. The molecules identified in sperm from multiple organisms were put together in the scheme. The cholesterol efflux shown at the left is suggested to participate in changes of membrane fluidity at capacitation in mammals. Sperm-activating peptide (SAP) binds directly or via a receptor to transmembrane guanylyl cyclase (mGC) and activates it. Nitric oxide (NO) activates a soluble guanylyl cyclase (sGC). cGMP activates a specific type of  $K^+$  channel, and consequently the membrane potential is hyperpolarized. Membrane hyperpolarization stimulates several voltage-gated channels or ion exchangers. The  $Na^+/H^+$  exchanger induces intracellular alkalinization, which raises enzymatic activity of dynein.  $Ca^{2+}$  efflux through the  $Na^+/Ca^{2+}$  exchangers plays a role in keeping intracellular  $Ca^{2+}$  low in sea urchin sperm. In fish, the converse action of this channel causes  $Ca^{2+}$  influx. In *Ciona* and salmonid fish, the production of cAMP is closely coupled with  $K^+$ -dependent membrane hyperpolarization. In carp, hyperpolarization activates  $Ca^{2+}$ -channels to cause  $Ca^{2+}$  influx. Transmembrane adenylyl cyclase (mAC) regulated by CaM was identified in sea urchin sperm. Soluble adenylyl cyclase is activated by bicarbonate ( $HCO_3^-$ ), which may be transported into the cell by  $Na^+/HCO_3^-$  cotransporter in mammals. The amounts of both cGMP and cAMP are negatively regulated by phosphodiesterases (PDEs). Some  $Ca^{2+}$ -channels in sperm are regulated by cAMP. Steroids, such as progesterone, induce  $Ca^{2+}$  influx possibly through its hypothetical receptor (R). Release of  $Ca^{2+}$  from store-operated  $Ca^{2+}$  channels is likely to be induced by  $IP_3$  produced by phospholipase C (PLC), which is activated by binding G protein (G). Protein phosphorylation by cAMP-dependent protein kinase (PKA), as well as by protein tyrosine kinase (PTK), modulates flagellar protein to activate motility. Increases in intracellular  $Ca^{2+}$  cause the activation of other channels or signaling molecules, as well as modulation of flagellar wave asymmetry, which causes changes in the direction of movement and ultimately leads to chemotaxis of sperm toward the egg.

and Shapiro, 1985) and is not discussed in detail in this review. The latter involves a signaling pathway from the plasma membrane to the axoneme. Several kinds of ionic channels and receptors along with the enzymes for cyclic nucleotide synthesis have been identified as molecules involved in this signaling pathway (Fig. 4). The final target of this signaling pathway is the alternation of axonemal movement, including activation of dynein by protein phosphorylation and modulation of flagellar bend asymmetry.

#### Receptors for extracellular sperm-activating substances

The egg jelly-associated peptide in the sea urchins *Hemicentrotus pulcherrimus* and *Strongylocentrotus purpuratus*, called speract (or sperm activating peptide I; SAP-I), is a peptide consisting of ten amino acids. This molecule binds to a specific sperm surface receptor, resulting in increased sperm motility and respiration rate (Suzuki *et al.*, 1981; Hansbrough and Garbers, 1981). Receptors for the

peptide were identified on the sperm membrane in *H. purpuratus* (Shimizu *et al.*, 1994) and in *S. purpuratus* (Dangott and Garbers, 1984) with molecular masses of 71 kDa and 77 kDa, respectively. The binding of speract to the receptor activates a guanylyl cyclase on the plasma membrane (Bentley *et al.*, 1988). A 14-amino acid peptide, resact (SAPIIA), from another species of sea urchin, *Arbacia punctulata*, binds directly to guanylyl cyclase on the sperm plasma membrane (Suzuki *et al.*, 1984; Singh *et al.*, 1988). Likewise, asterosap, a sperm-activating peptide from starfish, binds to a 130-kDa membrane protein that is likely to be a guanylyl cyclase (Nishigaki *et al.*, 2000).

Two proteins that activate sperm motility have been identified in herring: one has a small molecular mass (~8 kDa) (Oda *et al.*, 1998), while the other is a 105-kDa glycoprotein present on the micropyle of the egg (Vines *et al.*, 2002). Receptors for these two proteins have not been identified. Interestingly, chordates might have developed the use



of steroids as molecules to trigger modulation of sperm motility. An oocyte maturation-inducing hormone,  $17\alpha, 20\beta$ -dihydroxy-4-pregnen-3-one, is responsible for the maturation of sperm motility in the sperm duct of salmonid fish (Miura *et al.*, 1992). In the ascidians *Ciona intestinalis* and *C. savignyi*, a novel sulfate steroid, SAAF (sperm-activating and attracting factor), induces sperm activation and chemotaxis, but the receptor on sperm remains to be identified (Yoshida *et al.*, 2002). Progesterone activates sperm motility in mammals by inducing  $\text{Ca}^{2+}$  influx (Uhler *et al.*, 1992). On the other hand, sperm appear to receive signals in a manner similar to chemical reception of sensory organs. For example, specific olfactory receptors are expressed on the mid-piece of sperm flagella (Vanderhaeghen *et al.*, 1993). Recently, an odorant receptor, hOR17-4, was shown to be involved in the chemotaxis of human sperm (Spehr *et al.*, 2003).

### Ion channels and cyclic nucleotide cyclases

Binding of egg-derived substances to the sperm receptor or several kinds of ionic changes around sperm induce membrane hyperpolarization,  $\text{Ca}^{2+}$  influx, increases in intracellular pH, and activation of several enzymes related to the subsequent signal cascade for activation of sperm motility (Fig. 4). The membrane proteins involved in this process include voltage-dependent or cGMP-gated  $\text{K}^+$  channels,  $\text{Na}^+/\text{H}^+$  and  $\text{Na}^+/\text{Ca}^{2+}$  exchangers, and  $\text{Na}^+/\text{HCO}_3^-$  cotransporters. There is accumulating evidence for the presence of several ion channels on sperm, but only a few of these channels have been characterized well in relation to flagellar motility at the molecular level. Following the reception of extracellular signals, cyclic nucleotide cyclases are activated to induce transient increases in cGMP and cAMP, both of which are essential for intracellular signal transduction to activate axonemal movement.  $\text{Ca}^{2+}$  influx from outside the sperm through  $\text{Ca}^{2+}$  channels or the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger, or from intracellular stores through store-operated  $\text{Ca}^{2+}$ -channels (SOC) causes the activation of other channels or enzymes, as well as the changes in flagellar wave asymmetry necessary for chemotaxis toward the egg.

**$\text{K}^+$  channels:** A decrease in extracellular  $\text{K}^+$  concentration triggers the initiation of sperm motility in salmonid fish. In sea- or freshwater fish, such as carp, a change in osmolality triggers the initiation of sperm motility (Morisawa, 1994).  $\text{K}^+$ -dependent membrane hyperpolarization is caused by spawning into different ionic conditions in both cases (Takai and Morisawa, 1995; Kho *et al.*, 2001; Krasznai *et al.*, 2000). Membrane hyperpolarization has also been observed in *Ciona* sperm in which  $\text{K}^+$  permeability is increased by SAAF (Izumi *et al.*, 1999). Membrane hyperpolarization induces an increase in intracellular  $\text{Ca}^{2+}$  concentration and the activation of adenylyl cyclase (Kho *et al.*, 2001; Izumi *et al.*, 1999), which induces a transient increase in intracellular cAMP concentration. The  $\text{K}^+$  channel has not yet been isolated from teleost or *Ciona* sperm, but it may be similar to

the channel reported in *Paramecium* (Shultz *et al.*, 1992; Izumi *et al.*, 1999).

Intracellular levels of cyclic nucleotides increase at activation of sperm motility in most animals. Following the activation of guanylyl cyclase (GC) by binding of SAP, cGMP is synthesized and causes activation of a cGMP-gated  $\text{K}^+$  channel, resulting in  $\text{K}^+$  efflux and membrane hyperpolarization in sea urchin sperm (Babcock *et al.*, 1992; Galindo *et al.*, 2000). A cation channel, SPIH, has been cloned in sea urchin and shown to belong to the hyperpolarization-activated and cyclic nucleotide-gated  $\text{K}^+$  channel (HCN) family (Gauss *et al.*, 1998). SPIH has six putative transmembrane helices, a pore region, and cyclic nucleotide binding sites. SPIH is localized along the flagellum as a ~97-kDa phosphorylated form and may play roles in the activation of sperm motility.

**Guanylyl cyclase:** Transmembrane guanylyl cyclase (mGC) is a homodimeric glycoprotein. Peptides released from the egg, *i.e.*, resact in sea urchin and asterosap in starfish, can bind to a guanylyl cyclase to induce cGMP synthesis. In *Arbacia* sperm, binding of speract to the receptor activates guanylyl cyclase, resulting in cGMP production. Some phosphatases or phosphodiesterases may be involved in the rapid inactivation of GC or cGMP, respectively (Garbers, 1989; Suzuki, 1995). Some forms of guanylyl cyclase recognize atrial natriuretic peptides (ANP) (Garbers, 1991). Specific binding of ANP was detected in human sperm (Silvestroni *et al.*, 1992).

The soluble form of guanylyl cyclase (sGC) is a heterodimeric hemoprotein consisting of  $\alpha$  and  $\beta$  subunits. sGC is mainly activated by nitric oxide (NO) present in the female reproductive tract in the mouse (Burnett *et al.*, 1995). Sperm also express NO synthase (NOS) activity and synthesize NO by utilizing several NO donors (Rosselli *et al.*, 1996; Revelli *et al.*, 2002).

**Adenylyl cyclase:** Intracellular cAMP is synthesized by adenylyl cyclase (AC). Cell membrane-bound AC with transmembrane domains (mAC) is regulated by G protein or  $\text{Ca}^{2+}/\text{CaM}$  and has been characterized extensively in somatic cells. In sea urchins, a 190-kDa CaM-associated adenylyl cyclase was identified and shown to be localized mainly on the proximal half of the flagellum (Bookbinder *et al.*, 1990). Some types of  $\text{G}\alpha$  subunit are apparently localized in the flagella of mammalian sperm and may be involved in the modulation of adenylyl cyclase activity (Baxendale and Fraser, 2003). In human sperm, a CaM-dependent phosphodiesterase (PDE) is localized along the flagellum and seems to be involved in the regulation of flagellar cAMP level (Lefievre *et al.*, 2002).

Bicarbonate ( $\text{HCO}_3^-$ ) is involved in maturation of sperm motility in salmonid fish and mammals (Morisawa and Morisawa, 1988; Okamura *et al.*, 1985), although it has been shown to inhibit sperm motility in flatfish through the action of carbonic anhydrase (Inaba *et al.*, 2003). Recently,

a unique soluble adenylyl cyclase (sAC) was cloned and shown to be activated in a bicarbonate-dependent, G protein-independent manner (Buck *et al.*, 1999). As sperm AC is activated by extracellular bicarbonate ions (Okamura *et al.*, 1985), production of cAMP in sperm is likely to be carried out, at least in part, by soluble AC. A  $\text{Na}^+/\text{HCO}_3^-$  cotransporter is present in mouse sperm and could participate in the transport of  $\text{HCO}_3^-$  into sperm (Demarco *et al.*, 2003). Sperm hyperactivation appears to be modulated by reactive oxygen species (ROS) (Aitken and Fisher, 1994; de Lamirande *et al.*, 1997). The target of ROS is unclear, but has suggested to be a factor involved in cAMP production, most likely adenylyl cyclase (de Lamirande and Gagnon, 1999).

**$\text{Na}^+/\text{H}^+$  and  $\text{Na}^+/\text{Ca}^{2+}$  exchangers:** Influx of  $\text{Na}^+$  is essential for the motility of sea urchin sperm. The spawning of sperm into seawater involves intracellular alkalinization through the  $\text{Na}^+/\text{H}^+$  antiport that may induce activation of motility (Nishioka and Cross, 1978; Christen *et al.*, 1983). A voltage-sensitive  $\text{Na}^+/\text{H}^+$  exchanger has been suggested to be activated by  $\text{K}^+$ -dependent membrane hyperpolarization. Efflux of  $\text{H}^+$  induces intracellular alkalinization, which activates dyneins (Christen *et al.*, 1983; Johnson *et al.*, 1983; Lee, 1985). On the other hand, a 90-kDa  $\text{K}^+$ -dependent  $\text{Na}^+/\text{Ca}^{2+}$  exchanger, suNCKX, was identified and cloned in sea urchin (Su and Vacquier, 2002). This molecule is localized on the plasma membrane of sperm flagella and may maintain a low level of intracellular  $\text{Ca}^{2+}$ . It possesses two PKA sites and a His-rich region in the cytoplasmic loop, suggesting regulation of the channel by cAMP-dependent phosphorylation and metal binding. When herring sperm initiate motility upon reception of the 105-kDa glycoprotein, SMIF, membrane hyperpolarization occurs and subsequently both voltage-dependent  $\text{Ca}^{2+}$  channels and  $\text{Na}^+/\text{Ca}^{2+}$  exchangers appear to be activated for  $\text{Ca}^{2+}$  influx (Vines *et al.*, 2002). In this case, the activity of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger should operate in reverse. A putative 120-kDa  $\text{Na}^+/\text{Ca}^{2+}$  exchanger is localized along the flagella of herring sperm (Vines *et al.*, 2002).

**$\text{Ca}^{2+}$  channels:** Influx of external  $\text{Ca}^{2+}$  is known to be essential for activation of sperm motility. Several types of  $\text{Ca}^{2+}$  channels are involved in this process (Darszon *et al.*, 2001). A cAMP-regulated  $\text{Ca}^{2+}$  channel was identified and suggested to participate in  $\text{Ca}^{2+}$ -dependent depolarization in sea urchin sperm (Cook and Babcock, 1993). A putative 686-amino acid sperm cation channel, CatSper, has been isolated and cloned. CatSper is located in the principal piece of the flagella in mouse sperm and is involved in the regulation of sperm motility by mediating cAMP-induced  $\text{Ca}^{2+}$  influx (Ren *et al.*, 2001). In carp sperm, membrane hyperpolarization removes inactivation of  $\text{Ca}^{2+}$  channels and causes  $\text{Ca}^{2+}$  influx at initiation of sperm motility. However, the process of the activation of motility seems to be independent of cAMP (Krasznai *et al.*, 2000). On the other hand, homologs of *Drosophila* transient receptor potential channels (Trp) are

present in mouse sperm. Among them, Trp1 and Trp3 are localized on sperm flagella, suggesting that Trps are involved in the  $\text{Ca}^{2+}$  influx required for activation of sperm motility (Trevino *et al.*, 2001).

$\text{Ca}^{2+}$  release from intracellular stores is involved in the modulation of sperm motility. Store-operated  $\text{Ca}^{2+}$  channels have been shown to be involved in hyperactivation of bull sperm (Ho and Suarez, 2001) and chemotaxis in *Ciona* sperm (Yoshida *et al.*, 2003). Receptors for inositol 1,4,5-trisphosphate ( $\text{IP}_3$ ), which releases  $\text{Ca}^{2+}$  from intracellular stores, are localized on the flagella of mammalian sperm (Ho and Suarez, 2001a), suggesting that the  $\text{Ca}^{2+}$ -release signal for hyperactivation of sperm is  $\text{IP}_3$ .

### Lipid raft as a potential signaling scaffold

There is accumulating evidence that compartmentalization of the sperm plasma membrane is closely associated with the regulation of sperm motility. Albumin-induced sperm capacitation accompanies the decrease in sterol level of the plasma membrane. The similarity of the effect of albumin to those of high-density lipoprotein (HDL) and  $\beta$ -cyclodextrins suggests that serum albumin affects sperm capacitation through cholesterol efflux (Travis and Kopf, 2002). In fact, a loss of cholesterol was observed from the sperm plasma membrane and appears to be associated with cAMP-dependent phosphorylation and with tyrosine phosphorylation of sperm proteins (Visconti *et al.*, 1999).

The membrane subdomain rich in cholesterol and sphingolipids is called the "lipid raft." Efflux of cholesterol during sperm capacitation may be related to the dynamics of the membrane lipid raft by increasing membrane fluidity (Trevis and Kopf, 2002), and ultimately the dynamics of signal molecules in the raft, such as receptors and ion channels. For example, cholesterol efflux induces an increase in protein tyrosine phosphorylation in mammalian sperm (Visconti *et al.*, 1999). In addition, Trp1 is co-localized with caveolin-1, which is a major component of caveolae, a subset of the lipid raft (Trevino *et al.*, 2001). Bicarbonate, cAMP, and  $\text{Ca}^{2+}$  were reported to affect cholesterol depletion and lipid architecture, suggesting the rearrangement of membrane proteins upon activation of motility (Harrison *et al.*, 1996; Flesch *et al.*, 2001).

### III. Genome-wide and proteomic approaches to comprehensive understanding of flagellar architecture

The system of flagellar motility is composed of a sophisticated protein network. As described above, only a subpopulation of sperm flagellar proteins have been characterized at the molecular level. The relationship between each protein and the spatial organization of each component have not been elucidated. The flagellum contains more than 400 proteins, less than half of which have been characterized. To understand the architecture and function of flagellar motility, extensive analyses of flagellar proteins are required.

Recent genome-wide and proteomic studies have made

it possible to extensively describe proteins expressed in a given type of cell. For example, immunoscreening of cDNAs by antibodies against axonemal proteins revealed 76 axonemal proteins, including novel proteins, in *Ciona intestinalis* (Padma *et al.*, 2003). Mass spectrometric analysis in conjunction with two-dimensional gel electrophoresis identified a number of proteins in human airway cilia (Ostrowski *et al.*, 2002). Analysis of tyrosine phosphorylated proteins in human sperm by tandem mass spectrometry identified proteins that are phosphorylated during capacitation (Ficarro *et al.*, 2003). Application of these proteomic methods to analyses of flagellar compartments, such as the axoneme, plasma membrane, or lipid raft, may improve our overall understanding of how sperm cells are constructed and how the signaling network is involved in the rapid response of flagellar motility to changes in the ionic environment or to substances released from the egg.

## ACKNOWLEDGMENTS

The author thanks Yuhkoh Satouh for cooperation in drawing Fig. 2. The analysis of flagellar structure by EM was supported in part by the NIBB Cooperative Research Program (No. 3–133). The English editing of the manuscript by Dolphin Translation Co., Ltd is acknowledged. This work was supported in part by a grant from Intelligent Cosmos Academic Foundation and by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

## REFERENCES

- Adams GM, Huang B, Piperno G, Luck DJ (1981) Central-pair microtubular complex of *Chlamydomonas* flagella: polypeptide composition as revealed by analysis of mutants. *J Cell Biol* 91: 69–76
- Agustin JT, Wilkerson CG, Witman GB (2000) The unique catalytic subunit of sperm cAMP-dependent protein kinase is the product of an alternative  $\alpha$  mRNA expressed specifically in spermatogenic cells. *Mol Biol Cell* 11: 3031–3044
- Aitken J, Fisher H (1994) Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. *BioEssays* 16: 259–267
- Ashizawa K, Sakuragi M, Tsuzuki Y (1998) Temperature-dependent flagellar motility of demembranated, cytosol-free fowl spermatozoa. *Comp Biochem Physiol A* 121: 83–89
- Babcock DF, Bosma MM, Battaglia DE, Darszon A (1992) Early persistent activation of sperm  $K^+$  channels by the egg peptide speract. *Proc Natl Acad Sci USA* 89: 6001–6005
- Baccetti B, Afzelius BA (1976) The Biology of the Sperm Cell. Monographs in Developmental Biology, vol. 10, S. Karger Press, Basel
- Bannai H, Yoshimura M, Takahashi K, Shingyoji C (2000) Calcium regulation of microtubule sliding in reactivated sea urchin sperm flagella. *J Cell Sci* 113: 831–839
- Baxendale RW, Fraser LR (2003) Immunolocalization of multiple  $G\alpha$  subunits in mammalian spermatozoa and additional evidence for  $G\alpha_s$ . *Mol Reprod Dev* 65: 104–113
- Bentley JK, Khatra AS, Garbers DL (1988) Receptor-mediated activation of detergent-solubilized guanylate cyclase. *Biol Reprod* 39: 639–647
- Bernstein M, Beech PL, Katz SG, Rosenbaum, JL (1994) A new kinesin-like protein (Klp1) localized to a single microtubule of the *Chlamydomonas* flagellum. *J Cell Biol* 125: 1313–1326
- Berruti G, Porzio S (1990) Evidence for a calsequestrin-like calcium-binding protein in human spermatozoa. *Eur J Cell Biol* 52: 117–122
- Bookbinder LH, Moy GW, Vacquier VD. (1990) Identification of sea urchin sperm adenylate cyclase. *J Cell Biol* 111: 1859–1866
- Brokaw CJ (1979) Calcium-induced asymmetrical beating of triton-demembranated sea urchin sperm flagella. *J Cell Biol* 82: 401–411
- Buck J, Sinclair ML, Schapal L, Cann MJ, Levin LR (1999) Cytosolic adenylate cyclase defines a unique signaling molecule in mammals. *Proc Natl Acad Sci USA* 96: 79–84
- Burgess SA, Walker ML, Sakakibara H, Knight PJ, Oiwa K (2003) Dynein structure and power stroke. *Nature* 421: 715–718
- Burnett AL, Ricker DD, Chamness SL, Maguire MP, Crone JK, Bredt DS, Snyder SH, Chang TS (1995) Localization of nitric oxide synthase in the reproductive organs of the male rat. *Biol Reprod* 52: 1–7
- Carrera A, Gerton GL, Moss SB (1994) The major fibrous sheath polypeptide of mouse sperm: structural and functional similarities to the A-kinase anchoring proteins. *Dev Biol* 165: 272–284
- Casey DM, Inaba K, Pazour GJ, Takada S, Wakabayashi K, Wilkerson CG, Kamiya R, Witman GB (2003) DC3, the 21-kD subunit of the outer dynein arm-docking complex (ODA-DC), is a novel EF-hand protein important for assembly of both the outer arm and the ODA-DC. *Mol Biol Cell*, in press
- Chaudhry PS, Newcomer PA, Casillas ER (1991) Casein kinase I in bovine sperm: purification and characterization. *Biochem Biophys Res Commun* 179: 592–598
- Christen R, Schackmann RW, Shapiro BM (1983) Metabolism of sea urchin sperm. Interrelationships between intracellular pH, ATPase activity, and mitochondrial respiration. *J Biol Chem* 258: 5392–5399
- Cook SP, Babcock DF (1993) Activation of  $Ca^{2+}$  permeability by cAMP is coordinated through the pHi increase induced by speract. *J Biol Chem* 268: 22408–22413
- Cosson, MP (1990) Sperm chemotaxis, In: “Controls of Sperm Motility: Biological and Clinical Aspects”, Ed by C Gagnon, CRC Press, Boca Raton, FL, pp 103–135
- Dangott LJ, Garbers DL (1984) Identification and partial characterization of the receptor for speract. *J Biol Chem* 259: 13712–13716
- Darszon A, Beltran C, Felix R, Nishigaki T, Trevino CL (2001) Ion transport in sperm signaling. *Dev Biol* 240: 1–14
- de Lamirande E, Jiang H, Zini A, Kodama H, Gagnon C (1997) Reactive oxygen species and sperm physiology. *Rev Reprod* 2: 48–54
- de Lamirande E, Gagnon C (1999) The dark and bright sides of reactive oxygen species on sperm function. In “The Male gamete: From basic science to clinical application” Ed by C Gagnon, Cache River Press, IL, USA, pp 455–467
- Demarco IA, Espinosa F, Edwards J, Sosnik J, De La Vega-Beltran JL, Hockensmith JW, Kopf GS, Darszon A, Visconti PE (2003) Involvement of a  $Na^+/HCO_3^-$  cotransporter in mouse sperm capacitation. *J Biol Chem* 278: 7001–7009
- Dey CS, Brokaw CJ (1991) Activation of *Ciona* sperm motility: phosphorylation of dynein polypeptides and effects of a tyrosine kinase inhibitor. *J Cell Sci* 100: 815–824
- Dutcher SK. (2001) The tubulin fraternity: alpha to eta. *Curr Opin Cell Biol* 13: 49–54
- Dutcher SK, Huang B, Luck DJ (1984) Genetic dissection of the central pair microtubules of the flagella of *Chlamydomonas reinhardtii*. *J Cell Biol* 98: 229–236
- Eddy EM, Toshimori K, O'Brien DA (2003) Fibrous sheath of mammalian spermatozoa. *Microsc Res Tech* 61: 103–115
- Ficarro S, Chertihin O, Westbrook VA, White F, Jayes F, Kalab P, Marto JA, Shabanowitz J, Herr JC, Hunt DF, Visconti PE (2003)

- Phosphoproteome analysis of capacitated human sperm. Evidence of tyrosine phosphorylation of a kinase-anchoring protein 3 and valosin-containing protein/p97 during capacitation. *J Biol Chem* 278: 11579–11589
- Flesch FM, Brouwers JF, Nievelstein PF, Verkleij AJ, van Golde LM, Colenbrander B, Gadella BM (2001) Bicarbonate stimulated phospholipid scrambling induces cholesterol redistribution and enables cholesterol depletion in the sperm plasma membrane. *J Cell Sci* 114: 3543–3555
- Fujita A, Nakamura K, Kato T, Watanabe N, Ishizaki T, Kimura K, Mizoguchi A, Narumiya S (2000) Ropporin, a sperm-specific binding protein of rhophilin, that is localized in the fibrous sheath of sperm flagella. *J Cell Sci* 113: 103–112
- Gagnon C, White D, Cosson J, Huitorel P, Edde B, Desbruyeres E, Paturle-Lafanechere L, Multigner L, Job D, Cibert C (1996) The polyglutamylated lateral chain of alpha-tubulin plays a key role in flagellar motility. *J Cell Sci* 109: 1545–1553
- Gaillard AR, Diener DR, Rosenbaum JL, Sale WS (2001) Flagellar radial spoke protein 3 is an A-kinase anchoring protein (AKAP). *J Cell Biol* 153: 443–448
- Galindo BE, Beltran C, Cragoe EJ Jr, Darszon A (2000) Participation of a K<sup>+</sup> channel modulated directly by cGMP in the speract-induced signaling cascade of *Strongylocentrotus purpuratus* sea urchin sperm. *Dev Biol* 221: 285–294
- Garbers DL (1989) Molecular basis of fertilization. *Ann Rev Biochem* 58: 719–742
- Garbers DL (1991) Identification of a cell surface receptor common to germ and somatic cells. *Biol Reprod* 44: 225–230
- Gardner LC, O'Toole E, Perrone CA, Giddings T, Porter ME (1994) Components of a "dynein regulatory complex" are located at the junction between the radial spokes and the dynein arms in *Chlamydomonas* flagella. *J Cell Biol* 127: 1311–1325
- Gauss R, Seifert R, Kaupp UB (1998) Molecular identification of a hyperpolarization-activated channel in sea urchin sperm. *Nature* 393: 583–587
- Gee MA, Heuser JE, Vallee RB (1997) An extended microtubule-binding structure within the dynein motor domain. *Nature* 390: 636–639
- Gibbons IR (1981) Cilia and flagella of eukaryotes. *J Cell Biol* 91: 107s–124s
- Gibbons IR, Gibbons BH, Mocz G, Asai DJ (1991) Multiple nucleotide-binding sites in the sequence of dynein beta heavy chain. *Nature* 352: 640–643
- Gingras D, White D, Garin J, Cosson J, Huitorel P, Zingg H, Cibert C, Gagnon C (1998) Molecular cloning and characterization of a radial spoke head protein of sea urchin sperm axonemes: involvement of the protein in the regulation of sperm motility. *Mol Biol Cell* 9: 513–522
- Gingras D, White D, Garin J, Multigner L, Job D, Cosson J, Huitorel P, Zingg H, Dumas F, Gagnon C (1996) Purification, cloning, and sequence analysis of a Mr=30,000 protein from sea urchin axonemes that is important for sperm motility. Relationship of the protein to a dynein light chain. *J Biol Chem* 271: 12807–12813
- Goodenough UW, Heuser JE (1985) Substructure of inner dynein arms, radial spokes, and the central pair/projection complex of cilia and flagella. *J Cell Biol* 100: 2008–2018
- Habermacher G, Sale WS (1997) Regulation of flagellar dynein by phosphorylation of a 138-kD inner arm dynein intermediate chain. *J Cell Biol* 136: 167–176
- Hansbrough JR, Garbers DL (1981) Speract. Purification and characterization of a peptide associated with eggs that activates spermatozoa. *J Biol Chem* 256: 1447–1452
- Harrison RA, Ashworth PJ, Miller NG (1996) Bicarbonate/CO<sub>2</sub>, an effector of capacitation, induces a rapid and reversible change in the lipid architecture of boar sperm plasma membranes. *Mol Reprod Dev* 45: 378–391
- Hayashi H, Yamamoto K, Yonekawa H, Morisawa M (1987) Involvement of tyrosine protein kinase in the initiation of flagellar movement in rainbow trout spermatozoa. *J Biol Chem* 262: 16692–16698
- Henson JH, Cole DG, Roesener CD, Capuano S, Mendola RJ, Scholey JM (1997) The heterotrimeric motor protein kinesin-II localizes to the midpiece and flagellum of sea urchin and sand dollar sperm. *Cell Motil Cytoskeleton* 38: 29–37
- Herrmann GB, Koschorz B, Wertz K, McLaughlin KJ, Kispert A (1999) A protein kinase encoded by the t complex responder gene causes non-mendelian inheritance. *Nature* 402: 141–146
- Hinchcliffe EH, Linck RW (1998) Two proteins isolated from sea urchin sperm flagella: structural components common to the stable microtubules of axonemes and centrioles. *J Cell Sci* 111: 585–595
- Ho HC, Suarez SS (2001) An inositol 1,4,5-trisphosphate receptor-gated intracellular Ca<sup>2+</sup> store is involved in regulating sperm hyperactivated motility. *Biol Reprod* 65: 1606–1615
- Huitorel P, Audebert S, White D, Cosson J, Gagnon C (1999). Role of tubulin epitopes in the regulation of flagellar motility. In "The Male Gamete: From basic science to clinical application", Ed by C Gagnon, Cache River Press, IL, USA, pp 475–491
- Ikeda K, Brown JA, Yagi T, Norrander JM, Hirono M, Eccleston E, Kamiya R, Linck RW (2003) Rib72, a conserved protein associated with the ribbon compartment of flagellar A-microtubules and potentially involved in the linkage between outer doublet microtubules. *J Biol Chem* 278: 7725–7734
- Inaba K (2000) Conformational changes of dynein: mapping and sequence analysis of ATP/Vanadate-dependent trypsin-sensitive sites on the outer arm dynein  $\beta$  heavy chain from sea urchin sperm flagella. *J Biochem (Tokyo)* 127: 1115–1120
- Inaba K (2002) Dephosphorylation of Tctex2-related dynein light chain by type 2A protein phosphatase. *Biochem Biophys Res Commun* 297: 800–805
- Inaba K, Akazome Y, Morisawa M (1993) Purification of proteasomes from salmonid fish sperm and their localization along sperm flagella. *J Cell Sci* 104: 907–915
- Inaba K, Dreano C, Cosson J (2003) Control of flatfish sperm motility by CO<sub>2</sub> and carbonic anhydrase. *Cell Motil Cytoskeleton* 55: 174–187
- Inaba K, Kagami O, Ogawa K (1999) Tctex2-related outer arm dynein light chain is phosphorylated at activation of sperm motility. *Biochem Biophys Res Commun* 256: 177–183
- Inaba K, Mohri H (1989) Dynamic conformational changes of 21 S dynein ATPase coupled with ATP hydrolysis revealed by proteolytic digestion. *J Biol Chem* 264: 8384–8388
- Inaba K, Morisawa S, Morisawa M (1998) Proteasomes regulate the motility of salmonid fish sperm through modulation of cAMP-dependent phosphorylation of an outer arm dynein light chain. *J Cell Sci* 111: 1105–1115
- Inaba K, Padma P, Hozumi A (2002) Isolation of an inner arm dynein intermediate chain IC116 from *Ciona intestinalis* and its roles in flagellar motility. *Zool Sci* 19: 1435 (Abstract)
- Itoh A, Inaba K, Ohtake H, Fujinoki M, Morisawa M (2003) Characterization of cAMP-dependent protein kinase catalytic subunit from rainbow trout sperm. *Biochem Biophys Res Commun* 305: 855–861
- Izumi H, Marian T, Inaba K, Oka Y, Morisawa M (1999) Membrane hyperpolarization by sperm-activating and -attracting factor increases cAMP level and activates sperm motility in the ascidian *Ciona intestinalis*. *Dev Biol* 213: 246–256
- Jin ZX, Inaba K, Manaka K, Morisawa M, Hayashi H (1994) Monoclonal antibodies against the protein complex that contains the flagellar movement-initiating phosphoprotein of *Oncorhynchus keta*. *J Biochem (Tokyo)* 115: 885–890
- Johnson CH, Clapper DL, Winkler MM, Lee HC, Epel D (1983) A volatile inhibitor immobilizes sea urchin sperm in semen by

- depressing the intracellular pH. *Dev Biol* 98: 493–501
- Johnson KA, Haas MA, Rosenbaum JL (1994) Localization of a kinesin-related protein to the central apparatus of the *Chlamydomonas reinhardtii* flagellum. *J. Cell Sci* 6: 1551–1556
- Kalab P, Visconti P, Leclerc P, Kopf GS (1994) p95, the major phosphotyrosine-containing protein in mouse spermatozoa, is a hexokinase with unique properties. *J Biol Chem* 269: 3810–3817
- Kamiya R (2002) Functional diversity of axonemal dyneins as studied in *Chlamydomonas* mutants. *Int Rev Cytol* 219: 115–155
- Kato-Minoura T, Hirono M, Kamiya R (1997) *Chlamydomonas* inner-arm dynein mutant, ida5, has a mutation in an actin-encoding gene. *J Cell Biol* 137: 649–656
- King SJ, Dutcher SK. (1997) Phosphoregulation of an inner dynein arm complex in *Chlamydomonas reinhardtii* is altered in phototactic mutant strains. *J Cell Biol* 136: 177–191
- King SM (2000a) The dynein microtubule motor. *Biochim Biophys Acta* 1496: 60–75
- King SM (2000b) AAA domains and organization of the dynein motor unit. *J Cell Sci* 113: 2521–2526
- King SM, Marchese-Ragona SP, Parker SK, Detrich HW 3rd (1997) Inner and outer arm axonemal dyneins from the Antarctic rockcod *Notothenia coriiceps*. *Biochemistry* 36: 1306–1314
- Kho KH, Tanimoto S, Inaba K, Oka Y, Morisawa M (2001)  $Ca^{2+}$ /calmodulin-dependent membrane hyperpolarization increases cAMP to induce the initiation of sperm motility in rainbow trout, *Oncorhynchus mykiss*. *Zool Sci* 18: 919–928
- Koutoulis A, Pazour GJ, Wilkerson CG, Inaba K, Sheng H, Takada S, Witman GB (1997) The *Chlamydomonas reinhardtii* ODA3 gene encodes a protein of the outer dynein arm docking complex. *J Cell Biol* 137: 1069–1080
- Krasznai Z, Marian T, Izumi H, Damjanovich S, Balkay L, Tron L, Morisawa M (2000) Membrane hyperpolarization removes inactivation of  $Ca^{2+}$  channels, leading to  $Ca^{2+}$  influx and subsequent initiation of sperm motility in the common carp. *Proc Natl Acad Sci USA* 97: 2052–2057
- Kultgen PL, Byrd SK, Ostrowski LE, Milgram SL (2002) Characterization of an A-kinase anchoring protein in human ciliary axonemes. *Mol Biol Cell* 13: 4156–4166
- Lee HC (1985) The voltage-sensitive  $Na^+/H^+$  exchange in sea urchin spermatozoa flagellar membrane vesicles studied with an entrapped pH probe. *J Biol Chem* 260: 10794–10799
- Lefievre L, de Lamirande E, Gagnon C (2002) Presence of cyclic nucleotide phosphodiesterases PDE1A, existing as a stable complex with calmodulin, and PDE3A in human spermatozoa. *Biol Reprod* 67: 423–430
- Marshall WF, Rosenbaum JL (2000) How centrioles work: lessons from green yeast. *Curr Opin Cell Biol* 12: 119–125
- Miller RL (1985) Sperm chemo-orientation in the metazoa. In "Biology of Fertilization Vol. 2", Ed by CB Metz, A Monroy, Academic Press, New York, pp 275–337
- Mita M, Yasumasu I (1983) Metabolism of lipid and carbohydrate in sea urchin spermatozoa. *Gamete Res* 7: 133–144
- Mitchell DR (2000) *Chlamydomonas* flagella. *J Physiol* 36: 261–273
- Miura T, Yamauchi K, Takahashi H, Nagahama Y (1992) The role of hormones in the acquisition of sperm motility in salmonid fish. *J Exp Zool* 261: 359–363
- Mocz G, Gibbons IR (2001) Model for the motor component of dynein heavy chain based on homology to the AAA family of oligomeric ATPases. *Structure (Camb)* 9: 93–103
- Mohri H (1957) Endogenous substrates of respiration in sea urchin spermatozoa. *J Fac Sci Univ Tokyo Sec IV* 8: 51–63
- Mori C, Nakamura N, Welch JE, Gotoh H, Goulding EH, Fujioka M, Eddy EM (1998) Mouse spermatogenic cell-specific type 1 hexokinase (mHk1-s) transcripts are expressed by alternative splicing from the mHk1 gene and the HK1-S protein is localized mainly in the sperm tail. *Mol Reprod Dev* 49: 374–385
- Morisawa M. (1994) Cell signaling mechanisms for sperm motility. *Zool Sci* 11: 647–662
- Morisawa S, Morisawa M (1988) Induction of potential for sperm motility by bicarbonate and pH in rainbow trout and chum salmon. *J Exp Biol* 136: 13–22
- Nakano I, Kobayashi T, Yoshimura M, Shingyoji C (2003) Central-pair-linked regulation of microtubule sliding by calcium in flagellar axonemes. *J Cell Sci* 116: 1627–1636
- Nishigaki T, Chiba K, Hoshi M (2000) A 130-kDa membrane protein of sperm flagella is the receptor for asterosaps, sperm-activating peptides of starfish *Asterias amurensis*. *Dev Biol* 219: 154–162
- Nishioka D, Cross N (1978) The role of external sodium in sea urchin fertilization. In "Cell Reproduction", Ed by ER Dirksen, DN Prescottto, DF Fox, Academic Press, New York, pp 403–413
- Norrander JM, Perrone CA, Amos LA, Linck RW (1996) Structural comparison of tektins and evidence for their determination of complex spacings in flagellar microtubules. *J Mol Biol* 257: 385–397
- Oda S, Igarashi Y, Manaka K, Koibuchi N, Sakai-Sawada M, Sakai K, Morisawa M, Ohtake H, Shimizu N (1998) Sperm-activating proteins obtained from the herring eggs are homologous to trypsin inhibitors and synthesized in follicle cells. *Dev Biol* 204: 55–63
- Ogawa K (1994) Four ATP-binding sites in the midregion of the  $\beta$  heavy chain of dynein. *Nature* 352: 643–645
- Ogawa K, Takai H, Ogiwara A, Yokota E, Shimizu T, Inaba K, Mohri H (1996) Is outer arm dynein intermediate chain 1 multifunctional? *Mol Biol Cell* 7: 1895–1907
- Ogawa K, Kamiya R, Wilkerson CG, Witman GB (1995) Interspecies conservation of outer arm dynein intermediate chain sequences defines two intermediate chain subclasses. *Mol Biol Cell* 6: 685–696
- Okamura N, Tajima Y, Soejima A, Masuda H, Sugita Y (1985) Sodium bicarbonate in seminal plasma stimulates the motility of mammalian spermatozoa through direct activation of adenylate cyclase. *J Biol Chem* 260: 9699–9705
- Oko R (1988) Comparative analysis of proteins from the fibrous sheath and outer dense fibers of rat spermatozoa. *Biol Reprod* 39: 169–182
- Olds-Clarke P (1997) Models for male infertility: the t haplotypes. *Rev Reprod* 2: 157–164
- Olson GE, Sammons DW (1980) Structural chemistry of outer dense fibers of rat sperm. *Biol Reprod* 22: 319–332
- Ostrowski LE, Blackburn K, Radde KM, Moyer MB, Schlatter DM, Moseley A, Boucher RC (2002) A proteomic analysis of human cilia: identification of novel components. *Mol Cell Proteomics* 1: 451–465
- Padma P, Hozumi A, Ogawa K, Inaba K (2001) Molecular cloning and characterization of a thioredoxin/nucleoside diphosphate kinase related dynein intermediate chain from the ascidian, *Ciona intestinalis*. *Gene* 275: 177–183
- Padma P, Satouh Y, Wakabayashi K, Hozumi A, Ushimaru Y, Kamiya R, Inaba K (2003) Identification of a novel leucine-rich repeat protein as a component of flagellar radial spoke in the ascidian *Ciona intestinalis*. *Mol Biol Cell* 14: 774–785
- Patel-King RS, Benashski SE, King SM (2002) A bipartite  $Ca^{2+}$ -regulated nucleoside-diphosphate kinase system within the *Chlamydomonas* flagellum. The regulatory subunit p72. *J Biol Chem* 277: 34271–34279
- Perrone CA, Yang P, O'Toole E, Sale WS, Porter ME (1998) The *Chlamydomonas* IDA7 locus encodes a 140-kDa dynein intermediate chain required to assemble the I1 inner arm complex. *Mol Biol Cell* 9: 3351–3365
- Piperno G, Mead K, LeDizet M, Moscatelli A (1994) Mutations in the "dynein regulatory complex" alter the ATP-insensitive binding sites for inner arm dyneins in *Chlamydomonas* axonemes. *J*

- Cell Biol 125: 1109–1117
- Piperno G, Ramanis Z, Smith EF, Sale WS (1990) Three distinct inner dynein arms in *Chlamydomonas* flagella: molecular composition and location in the axoneme. J Cell Biol 110: 379–389
- Porter ME, Sale WS (2000) The 9+2 axoneme anchors multiple inner arm dyneins and a network of kinases and phosphatases that control motility. J Cell Biol 151: F37–F42
- Ren D, Navarro B, Perez G, Jackson AC, Hsu S, Shi Q, Tilly JL, Clapham DE (2001) A sperm ion channel required for sperm motility and male fertility. Nature 413: 603–609
- Revelli A, Ghigo D, Moffa F, Massobrio M, Tur-Kaspa, I (2002) Guanylate cyclase activity and sperm function. Endocrine Reviews 23: 484–494
- Rosenbaum JL, Witman GB (2002) Intraflagellar transport. Nat Rev Mol Cell Biol 3: 813–825
- Rosselli M, Dubey RK, Rosselli MA, Makas E, Fink D, Lauper U, Keller PJ, Imthurn B (1996) Identification of nitric oxide synthase in human and bovine oviduct. Mol Hum Reprod 2: 607–612
- Rupp G, O'Toole E, Porter ME (2001) The *Chlamydomonas* PF6 locus encodes a large alanine/proline-rich polypeptide that is required for assembly of a central pair projection and regulates flagellar motility. Mol Biol Cell 12: 739–751
- San Agustin JT, Leszyk JD, Nuwaysir LM, Witman GB (1998) The catalytic subunit of the cAMP-dependent protein kinase of ovine sperm flagella has a unique amino-terminal sequence. J Biol Chem 273: 24874–24883
- Schultz JE, Klumpp S, Benz R, Schurhoff-Goeters WJ, Schmid A (1992) Regulation of adenylyl cyclase from *Paramecium* by an intrinsic potassium conductance. Science 255: 600–603
- Sapiro R, Kostetskii I, Olds-Clarke P, Gerton GL, Radice GL, Strauss III JF (2002) Male infertility, impaired sperm motility, and hydrocephalus in mice deficient in sperm-associated antigen 6. Mol Cell Biol 22: 6298–6305
- Shao X, Tarnasky HA, Schalles U, Oko R, van der Hoorn FA (1997) Interactional cloning of the 84-kDa major outer dense fiber protein Odf84. Leucine zippers mediate associations of Odf84 and Odf27. J Biol Chem 272: 6105–6113
- Shimizu T, Yoshino K, Suzuki N (1994) Identification and characterization of putative receptor for sperm-activating peptide I (SAP-I) in spermatozoa of sea urchin *Hemicentrotus pulcherrimus*. Dev Growth Differ 36: 209–221
- Si Y, Okuno M (1995) Extrusion of microtubule doublet outer dense fibers 5-6 associating with fibrous sheath sliding in mouse sperm flagella. J Exp Zool 273: 355–362
- Si Y, Okuno M (1999) Role of tyrosine phosphorylation of flagellar proteins in hamster sperm hyperactivation. Biol Reprod 61: 240–246
- Silvestroni L, Palleschi S, Guglielmi R, Tosti Croce C (1992) Identification and localization of atrial natriuretic factor receptors in human spermatozoa. Arch Androl 28: 75–82
- Singh S, Lowe DG, Thorpe DS, Rodriguez H, Kuang WJ, Dangott LJ, Chinkers M, Goeddel DV, Garbers DL (1988) Membrane guanylate cyclase is a cell-surface receptor with homology to protein kinases. Nature 334: 708–712
- Smith EF (2002) Regulation of flagellar dynein by calcium and a role for an axonemal calmodulin and calmodulin-dependent kinase. Mol Biol Cell 13: 3303–3313
- Smith EF, Lefebvre PA (1996) PF16 encodes a protein with armadillo repeats and localizes to a single microtubule of the central apparatus in *Chlamydomonas* flagella. J Cell Biol 132: 359–370
- Smith EF, Lefebvre PA (1997a) PF20 gene product contains WD repeats and localizes to the intermicrotubule bridges in *Chlamydomonas* flagella. Mol Biol Cell 8: 455–467
- Smith EF, Lefebvre PA (1997b) The role of central apparatus components in flagellar motility and microtubule assembly. Cell Motil Cytoskeleton 38: 1–8
- Smrzka OW, Delgehyr N, Bornens M (2000) Tissue-specific expression and subcellular localisation of mammalian delta-tubulin. Curr Biol 10: 413–416
- Spehr M, Gisselmann G, Poplawski A, Riffell JA, Wetzel CH, Zimmer RK, Hatt H (2003) Identification of a testicular odorant receptor mediating human sperm chemotaxis. Science 299: 2054–2058
- Stephens RE (1970) Isolation of nexin – the linkage protein responsible for maintenance of the nine-fold configuration of flagellar axonemes. Biol Bull 139: 438
- Stephens RE, Lemieux NA (1999) Molecular chaperones in cilia and flagella: implications for protein turnover. Cell Motil Cytoskeleton 44: 274–283
- Stephens RE, Oleszko-Szuts S, Linck RW. (1989) Retention of ciliary ninefold structure after removal of microtubules. J Cell Sci 92: 391–402
- Su YH, Vacquier VD (2002) A flagellar K<sup>+</sup>-dependent Na<sup>+</sup>/Ca<sup>2+</sup> exchanger keeps Ca<sup>2+</sup> low in sea urchin spermatozoa. Proc Natl Acad Sci USA 99: 6743–6748
- Suzuki N, Nomura K, Ohtake H, Isaka S (1988) Purification and the primary structure of sperm-activity peptides from the jelly coat of sea urchin eggs. Biochem Biophys Res Commun 99: 1238–1244
- Suzuki N, Shimomura H, Radany EW, Ramarao CS, Ward GE, Bentley JK, Garbers DL (1984) A peptide associated with eggs causes a mobility shift in a major plasma membrane protein of spermatozoa. J Biol Chem 259: 14874–14879
- Suzuki N (1995) Structure, function and biosynthesis of sperm-activating peptides and fucose sulfate glycoconjugate in the extracellular coat of sea urchin eggs. Zool Sci 12: 13–27
- Takada S, Kamiya R (1994) Functional reconstitution of *Chlamydomonas* outer dynein arms from  $\alpha$ - $\beta$  and  $\gamma$  subunits: requirement of a third factor. J Cell Biol 126: 737–745
- Takada S, Wilkerson CG, Wakabayashi K, Kamiya R, Witman GB (2002) The outer dynein arm-docking complex: composition and characterization of a subunit (oda1) necessary for outer arm assembly. Mol Biol Cell 13: 1015–1029
- Takai H, Morisawa M (1995) Change in intracellular K<sup>+</sup> concentration caused by external osmolality change regulates sperm motility of marine and freshwater teleosts. J Cell Sci 108: 1175–1181
- Tash JS, Krinks M, Patel J, Means RL, Klee CB, Means AR (1988) Identification, characterization, and functional correlation of calmodulin-dependent protein phosphatase in sperm. J Cell Biol 106: 1625–1633
- Tombes RM, Shapiro BM (1985) Metabolite channeling: a phosphorylcreatine shuttle to mediate high energy phosphate transport between sperm mitochondrion and tail. Cell 41: 325–334
- Travis AJ, Kopf GS (2000) The role of cholesterol efflux in regulating the fertilization potential of mammalian spermatozoa. J Clin Invest 110: 731–736
- Trevino CL, Serrano CJ, Beltran C, Felix R, Darszon A (2001) Identification of mouse trp homologs and lipid rafts from spermatogenic cells and sperm. FEBS Lett 509: 119–125
- Ueno H, Gonda K, Takeda T, Numata O (2003) Identification of elongation factor-1 $\alpha$  as a Ca<sup>2+</sup>/calmodulin-binding protein in *Tetrahymena* cilia. Cell Motil Cytoskeleton 55: 51–60
- Uhler ML, Leung A, Chan SY, Wang C (1992) Direct effects of progesterone and antiprogesterone on human sperm hyperactivated motility and acrosome reaction. Fertil Steril 58: 1191–1198
- Vanderhaeghen P, Schurmans S, Vassart G, Parmentier M (1993) Olfactory receptors are displayed on dog mature sperm cells. J Cell Biol 123: 1441–1452
- Vera JC, Brito M, Zuvic T, Burzio LO (1984) Polypeptide composition of rat sperm outer dense fibers. A simple procedure to isolate the fibrillar complex. J Biol Chem 259: 5970–5977



- Vijayaraghavan S, Mohan J, Gray H, Khatra B, Carr DW (2000) A role for phosphorylation of glycogen synthase kinase-3 $\alpha$  in bovine sperm motility regulation. *Biol Reprod* 62: 1647–1654
- Vines CA, Yoshida K, Griffin FJ, Pillai MC, Morisawa M, Yanagimachi R, Cherr GN (2002) Motility initiation in herring sperm is regulated by reverse sodium-calcium exchange. *Proc Natl Acad Sci USA* 99: 2026–2031
- Visconti PE, Bailey JL, Moore GD, Pan D, Olds-Clarke P, Kopf GS (1995) Capacitation of mouse spermatozoa. I. Correlation between the capacitation state and protein tyrosine phosphorylation. *Development* 121: 1129–1137
- Visconti PE, Ning X, Fornes MW, Alvarez JG, Stein P, Connors SA, Kopf GS (1999) Cholesterol efflux-mediated signal transduction in mammalian sperm: cholesterol release signals an increase in protein tyrosine phosphorylation during mouse sperm capacitation. *Dev Biol* 214: 429–443
- Wargo MJ, Smith EF (2003) Asymmetry of the central apparatus defines the location of active microtubule sliding in *Chlamydomonas* flagella. *Proc Natl Acad Sci USA* 100: 137–142
- Wasco WM, Kincaid RL, Orr GA. (1989) Identification and characterization of calmodulin-binding proteins in mammalian sperm flagella. *J Biol Chem* 264: 5104–5111
- Woolley DM (1997) Studies on the eel sperm flagellum. I. The structure of the inner dynein arm complex. *J Cell Sci* 110: 85–94
- Yanagimachi R (1970) The movement of golden hamster spermatozoa before and after capacitation. *J Reprod Fertil* 23: 193–196
- Yang P, Fox L, Colbran RJ, Sale WS (2000) Protein phosphatases PP1 and PP2A are located in distinct positions in the *Chlamydomonas* flagellar axoneme. *J Cell Sci* 113: 91–102
- Yang P, Diener DR, Rosenbaum JL, Sale WS (2001) Localization of calmodulin and dynein light chain LC8 in flagellar radial spokes. *J Cell Biol* 153: 1315–1326
- Yang P, Sale WS (2000) Casein kinase I is anchored on axonemal doublet microtubules and regulates flagellar dynein phosphorylation and activity. *J Biol Chem* 275: 18905–18912
- Yang P, Sale WS (1998) The Mr 140,000 intermediate chain of *Chlamydomonas* flagellar inner arm dynein is a WD-repeat protein implicated in dynein arm anchoring. *Mol Biol Cell* 9: 3335–3349
- Yoshida M, Ishikawa M, Izumi H, De Santis R, Morisawa M (2003) Store-operated calcium channel regulates the chemotactic behavior of ascidian sperm. *Proc Natl Acad Sci USA* 100: 149–154
- Yoshida M, Murata M, Inaba K, Morisawa M (2002) A chemoattractant for ascidian spermatozoa is a sulfated steroid. *Proc Natl Acad Sci USA* 99: 14831–14836
- Zhang Z, Sapiro R, Kapfhamer D, Bucan M, Bray J, Chennathukuzhi V, McNamara P, Curtis A, Zhang M, Blanchette-Mackie EJ, Strauss JF 3rd (2002) A sperm-associated WD repeat protein orthologous to *Chlamydomonas* PF20 associates with Spag6, the mammalian orthologue of *Chlamydomonas* PF16. *Mol Cell Biol* 22: 7993–8004

(Accepted June 9, 2003 / Invited Review)