III-6 Cilia and Flagella

Keiichi Takahashi

*Zoological Institute, Faculty of The Science, University of Tokyo,
Hongo, Tokyo 113, Japan*

Mechanism of movement

1) The sliding microtubule model

The sliding microtubule model, first proposed in the mid-1960s, now seems to constitute the main framework in most of the studies on cilia and flagella. Direct evidence that an active sliding of microtubules causes a bending movement of flagella was obtained by Shingyoji, Murakami and Takahashi (1977) who used iontophoretic application of ATP to reactivate localized regions along the lengths of flagellar axonemes. This induced in the axoneme a pair of bends in exact conformity with the predictions of the sliding tubule model. Hiramoto and Baba (1978) analyzed the flagellar movement recorded with a high-speed camera and proposed, in order to explain the sine-generated nature of the flagellar waves they found, a model in which sliding activity is propagated along and around the flagellar axoneme. In a different approach, Sugino and Naitoh (1982) used computer simulation to obtain changing patterns of active sliding within a beating *Paramecium* cilia. Their model was successful in predicting the bending of the cilia in detail in both normal and high viscosity media.

2) Physical parameters

Okuno and Hiramoto (1979) measured the stiffness of echinoderm sperm flagella with a flexible glass microneedle and showed that the demembranated flagella were much stiffer in the absence than in the presence of ATP, probably due to the "rigor" crossbridges between the doublet microtubules.

The velocity of microtubule sliding has been determined in trypsin-treated axonemal fragments of the sea urchin spermatozoa under a dark field microscope. Miki-Noumura and her colleagues (Hata, Yano and Miki-Noumura, 1979; Yano and Miki-Noumura, 1980) and Takahashi, Shingyoji and Kamimura (1982) independently determined the ATP concentration dependency of the sliding velocity. The velocity was reduced by about 50% when the outer dynein arms were removed (Hata *et al.*, 1980; Yano and Miki-Noumura, 1981a) but showed considerable recovery after the arm-depleted axonemes were recombined with extracted dynein 1. These results indicated a functional similarity between the outer and inner dynein arms (Yano and Miki-Noumura, 1981a).

The force of microtubule sliding was measured by attaching a pair of glass microneedles to the axoneme of a sea urchin spermatozoon. In the presence of ATP and elastase the axoneme underwent sliding disintegration and, at the same time, exerted a pull on the microneedles. From the elastic bending of the microneedles the average force generated by a single dynein arm in ATP concentrations between 20 and 500 μ M was estimated at about 1 pN (Kamimura and Takahashi, 1981; Takahashi and Kamimura, 1982).

3) *Dynein*

Biochemical properties of dynein from *Tetrahymena* cilia were studied by Hoshino, and by Mabuchi and his co-workers, among others (reviewed by Mabuchi, 1978), and those of dynein from sea urchin sperm flagella by Ogawa and his co-workers (Ogawa and Mohri, 1972; Ogawa, 1973; Ogawa, Okuno and Mohri, 1975). The latter workers prepared antiserum against dynein 1 using its tryptic fragment, Fragment 1A (Ogawa and Mohri, 1975), which inhibited ATP-induced movement (Masuda, Ogawa and Miki-Noumura, 1978) and, to a lesser extent, the ATPase activity of demembrated sea urchin spermatozoa (Okuno, Ogawa and Mohri, 1976). Immunoelectron microscopy and other immunological techniques were used to reveal dissimilarities between the inner and outer arms (Ogawa, Mohri and Mohri, 1977; Ogawa, Negishi and Obika, 1982) although their functional implications remain to be elucidated.

4) *The dynein-arm crossbridge cycle*

Masami Takahashi and Tonomura (1978) found that 30S dynein from *Tetrahymena* cilia binds to either the A or B tubules of isolated doublets, but that the addition of 1 μ M ATP causes dissociation of the dynein from B tubules without affecting the binding of dynein to A tubules. This observation, suggesting that MgATP^{2-} causes detachment of the dynein crossbridges, was an important step in the study of the dynein arm crossbridge cycle. Other studies bearing on the nature of the crossbridge cycle include those on the inhibitory effects of vanadate on the motility and mechanical properties of sea urchin sperm flagella (Okuno, 1980). A detailed study of the nature of the inhibitory action of vanadate on ciliary dynein ATPase from *Tetrahymena* has been made by Shimizu (1981) who showed that the inhibition of 30S dynein ATPase by vanadate was noncompetitive, and that the inhibition was so potent that the kinetics of "tight-binding" inhibition was applicable to the system. Thus, the molecular weight of 420,000 to 480,000 per enzymatic active site was estimated with several assumptions.

In the currently controversial area of the morphology of dynein, Yano and Miki-Noumura (1981b) obtained electron microscopic images of negatively stained, isolated *Tetrahymena* dynein arms with a heart-like or Y-shaped appearance. They also found the distal region of the dynein arm to be split into two heads. On the other hand, Tsukita *et al.* (1983) used a rapid-freeze, deep-etch replica technique to observe the ATP-dependent changes in the structure of the arm in *Tetrahymena* cilia.

Control of movement

1) *Cilia and flagella of unicellular organisms*

Following the pioneering work of Kamada in the late 1920s, extensive studies have been made on the electrophysiology of ciliated protozoans by Kinosita and his students (Kinosita, 1979), who established that the cilia in these organisms are under the control of the cell membrane and that calcium ions play a key role in the control mechanism. In their classical experiments, Naitoh and Kaneko (1972) showed that demembrated "models" of *Paramecium* can be made to swim either forward (with normal ciliary beat) or backward (with "reversed" ciliary beat) by controlling the Ca^{2+} concentration in the reactivating solution containing Mg-ATP^{2-} . Localization of Ca-binding sites within cilia was studied by Tsuchiya and his co-workers (Tsuchiya and Takahashi, 1976; Tsuchiya, 1976; Tsuchiya and Suzuki, 1981) by using electron microscopy and electron probe microanalysis. Ogura and Takahashi (1976) found, by deciliation experiments, that the Ca-channels responsible for the Ca^{2+} action

potential and hence the ciliary reversal, are localized in the ciliary membrane.

Mihoko Takahashi (1979) obtained behavioural mutants from *Paramecium caudatum* which do not exhibit natural autogamy and therefore are more difficult for genetic studies than *Paramecium tetraurelia*. These mutants include those incapable of backward swimming and a slow swimmer. The former have defects in their membrane excitability (Takahashi and Naitoh, 1978). Some of the defects could be restored by microinjection of a soluble wild type gene product (Hiwatashi, Haga and Takahashi, 1980; Haga and Hiwatashi, 1982). The slow swimmer appears to have a normal membrane (Takahashi and Naitoh, 1978) but its dynein is altered (Hayashi and Takahashi, 1979). Behavioural mutants with alterations in the axonemes were also isolated from *Chlamydomonas* (Nakamura, 1981).

The mechanism by which the intracellular Ca^{2+} controls the ciliary movement is unknown. Mogami and Takahashi (1983) found that Ca^{2+} over a wide range of concentration has no effect on the sliding of doublet microtubules in trypsin-treated ciliary axonemes of *Paramecium*, which indicates that the Ca^{2+} sensitivity resides outside the basic sliding machinery. Miki-Noumura and Kamiya (1979) found that the doublets isolated from the sea urchin sperm flagella undergo a Ca-dependent conformational change, which they suggested might play a role in the control of movement.

Calmodulin was isolated from *Tetrahymena* by Watanabe and his colleagues (Suzuki *et al.*, 1979, 1981) and its intraciliary localization was demonstrated by indirect immunofluorescence (Suzuki *et al.*, 1982) and by immunoelectron microscopy (Ohnishi, Suzuki and Watanabe, 1982) which suggested the interdoublet links as calmodulin-binding sites. Although trifluoperazine stops the forward swimming of *Tetrahymena* and causes backward swimming in some of them (Suzuki *et al.*, 1982), the role of calmodulin in ciliary movement remains to be elucidated.

The activation of movement in spermatozoa has recently been given considerable attention. In fish sperm, the central role of the ionic environment in the maintenance of the quiescent state in the seminal plasma and in the initiation of movement following discharge has been revealed by Morisawa and Suzuki (1980). Using demembranated trout sperm, Morisawa and Okuno (1982) showed that cyclic AMP (cAMP) is required for the initiation of axonemal motility. In sea-urchin and starfish sperm, Ishiguro, Murofushi and Sakai, (1982) obtained evidence that cAMP-dependent protein kinase and a protein factor, both obtained from the detergent extract of the sperm, are necessary for reactivation of the Triton-extracted models and proposed that reactivation requires phosphorylation of the protein factor by the kinase.

2) Metazoan cilia

Most of the studies on the control of metazoan cilia have been carried out with the lateral cilia on the gill of the mussel, *Mytilus*. The ciliary arrest response in these cilia are mediated by membrane excitation (action potential) in the ciliated cell (Murakami and Takahashi, 1975a; Saimi, Murakami and Takahashi, 1983a, b). The response depends on the influx of Ca^{2+} through membrane channels (Motokawa, Murakami and Takahashi, 1975; Murakami and Takahashi, 1975b). An intraciliary Ca^{2+} concentration of above 10^{-7} – 10^{-6} M is required for the arrest as shown by reactivation experiments with demembranated cilia (Tsuchiya, 1977).

REFERENCES

- HAGA, H. and HIWATASHI, K. (1982). *Cell Biol. Int. Rep.* 6, 295–300.

- HATA, H., YANO, Y. and MIKI-NOUMURA, T. (1979). *Exp. Cell Res.* **122**, 416–419.
- HATA, H., YANO, Y., MOHRI, T., MOHRI, H. and MIKI-NOUMURA, T. (1980). *J. Cell Sci.* **41**, 331–340.
- HAYASHI, M. and TAKAHASHI, M. (1979). *J. Biol. Cehm.* **254**, 11561–11565.
- HIRAMOTO, Y. and BABA, S. A. (1978). *J. Exp. Biol.* **76**, 85–104.
- HIWATASHI, K., HAGA, N. and TAKAHASHI, M. (1980). *J. Cell Biol.* **84**, 476–480.
- ISHIGURO, K., MUROFUSHI, H. and SAKAI, H. (1982). *J. Cell Biol.* **92**, 777–782.
- KAMIMURA, S. and TAKAHASHI, K. (1981). *Nature* **293**, 566–568.
- KINOSITA, H. (1979). *Acta Protozool.* **18**, 23–24.
- MABUCHI, I. (1978). *Horizons Biochem. Biophys.* **5**, 1–36.
- MASUDA, H., OGAWA, K. and MIKI-NOUMURA, T. (1978). *Exp. Cell Res.* **115**, 435–439.
- MIKI-NOUMURA, T. and KAMIYA, R. (1979). *J. Cell Biol.* **81**, 355–360.
- MOGAMI, Y. and TAKAHASHI, K. (1983). *J. Cell Sci.* **61**, 107–121.
- MORISAWA, M. and OKUNO, M. (1982). *Nature* **295**, 703–704.
- MORISAWA, M. and SUZUKI, K. (1980). *Science* **210**, 1145–1147.
- MOTOKAWA, T., MURAKAMI, A. and TAKAHASHI, K. (1975). *J. Fac. Sci. Univ. Tokyo, IV* **13**, 243–249.
- MURAKAMI, A. and TAKAHASHI, K. (1975a). *Nature* **257**, 48–49.
- MURAKAMI, A. and TAKAHASHI, K. (1975b). *J. Fac. Sci. Univ. Tokyo, IV* **13**, 251–256.
- NAITOH, Y. and KANEKO, H. (1972). *Science* **176**, 523–524.
- NAKAMURA, S. (1981). *Cell Struct. Funct.* **6**, 385–393.
- OGAWA, K. (1973). *Biochim. Biophys. Acta* **293**, 514–525.
- OGAWA, K. and MOHRI, H. (1972). *Biochim. Biophys. Acta* **256**, 142–155.
- OGAWA, K. and MOHRI, H. (1975). *J. Biol. Chem.* **250**, 6476–6483.
- OGAWA, K., MOHRI, T. and MOHRI, H. (1977). *Proc. Natl. Acad. Sci. USA* **74**, 5006–5010.
- OGAWA, K., NEGISHI, S. and OBIKA, M. (1982). *J. Cell Biol.* **92**, 706–713.
- OGAWA, K., OKUNO, M. and MOHRI, H. (1975). *J. Biochem.* **78**, 729–737.
- OGURA, A. and TAKAHASHI, K. (1976). *Nature* **264**, 170–172.
- OHNISHI, K., SUZUKI, Y. and WATANABE, Y. (1982). *Exp. Cell Res.* **137**, 217–227.
- OKUNO, M. (1980). *J. Cell Biol.* **85**, 712–725.
- OKUNO, M. and HIRAMOTO, Y. (1979). *J. Exp. Biol.* **79**, 235–243.
- OKUNO, M., OGAWA, K. and MOHRI, H. (1976). *Biochem. Biophys. Res. Comm.* **68**, 901–906.
- SAIMI, Y., MURAKAMI, A. and TAKAHASHI, K. (1983a). *Comp. Biochem. Physiol.* **74A**, 499–506.
- SAIMI, Y., MURAKAMI, A. and TAKAHASHI, K. (1983b). *Comp. Biochem. Physiol.* **74A**, 507–511.
- SHIMIZU, T. (1981). *Biochemistry* **20**, 4347–4354.
- SHINGYOJI, C., MURAKAMI, A. and TAKAHASHI, K. (1977). *Nature* **265**, 269–270.
- SUGINO, K. and NAITOH, Y. (1982). *Nature* **295**, 609–611.
- SUZUKI, Y., HIRABAYASHI, T. and WATANABE, Y. (1979). *Biochem. Biophys. Res. Comm.* **90**, 253–260.
- SUZUKI, Y., NAGAO, S., ABE, K., HIRABAYASHI, T. and WATANABE, Y. (1981). *J. Biochem.* **89**, 333–336.
- SUZUKI, Y., OHNISHI, K., HIRABAYASHI, T. and WATANABE, Y. (1982). *Exp. Cell Res.* **137**, 1–14.
- TAKAHASHI, K. and KAMIMURA, S. (1982). in *Biological Functions of Microtubules and Related Structures*, eds. SAKAI, H., MOHRI, H. and BORISY, G. G., pp. 177–187.
- TAKAHASHI, K., SHINGYOJI, C. and KAMIMURA, S. (1982). *Symp. Soc. Exp. Biol.* **35**, 159–177.
- TAKAHASHI, M. and TONOMURA, Y. (1978). *J. Biochem.* **84**, 1339–1355.
- TAKAHASHI, M. (1979). *Genetics* **91**, 393–408.
- TAKAHASHI, M. and NAITOH, Y. (1978). *Nature* **271**, 656–659.
- TSUCHIYA, T. (1976). *Experientia* **32**, 1176–1177.
- TSUCHIYA, T. (1977). *Comp. Biochem. Physiol.* **56A**, 353–361.
- TSUCHIYA, T. and SUZUKI, S. (1981). *Experientia* **37**, 721–722.
- TSUCHIYA, T. and TAKAHASHI, K. (1976). *J. Protozool.* **23**, 523–526.
- TSUKITA, S., TSUKITA, S., USUKURA, J. and ISHIKAWA, H. (1983). *J. Cell Biol.* **96**, 1480–1485.
- YANO, Y. and MIKI-NOUMURA, T. (1980). *J. Cell Sci.* **44**, 169–186.
- YANO, Y. and MIKI-NOUMURA, T. (1981a). *J. Cell Sci.* **48**, 223–239.
- YANO, Y. and MIKI-NOUMURA, T. (1981b). *Biomed. Res.* **2**, 73–78.