Introduction - Background of studies of ciliary beating

Beating of cilia and eukaryotic flagella is the most basic locomotory movement of living creatures. In spite of their small size (in sea urchin cilia, diameter of 200nm and length of 30μm), they sufficiently act as locomotors in the aqueous environment of low Reynolds number. The terms, cilia and flagella, have conventionally been defined by differences in motile patterns of these organelles. However they have a common basic structure which is well known as axoneme of "9+2 structure" (Fig.I- 1). There are a central pair of singlet microtubules and nine outer doublet microtubules. Two projections from the A- tubule of each outer doublet are called outer and inner dynein arms, which undergo ATP- dependent cycles of attachment and detachment to the adjacent doublet microtubule. This causes sliding between outer doublet microtubules. The sliding movement is converted to bending motion. Bendings to the opposite sides, principal and reverse, alternately propagate along the axoneme from base to tip with an almost constant curvature in flagella. In cilia, bending grows at the base keeping the remaining part almost straight during the effective stroke and it propagates to the tip retaining the curvature during the recovery stroke. The precise mechanisms of the conversion from sliding to bending are still unknown.

It has been considered that cilia and flagella can generate two- or three-dimensional movements by converting uni-directionally sliding into bending. The aim of this research is to explore the mechanisms of the sliding-bending conversion. The variety of the movement of cilia and flagella might arise from the appropriate regulation

of sliding-bending conversion. For example, cilia of *Paramecium* can change the net direction of beating depending on the cellular excitability in response to the external stimuli (ciliary reversal). The reversal of the beating is known to be caused by integrating sliding movement of a common direction, i.e., the force generation of dynein arm is known to be preserved, from base to tip of the axoneme, even in the reversal response (Mogami and Takahashi, 1983).

Mechano-chemical aspect of the regulation of ciliary beating have been studied mainly on ciliate protozoan, such as *Paramecium* and *Tetrahymena*. Dense ciliation of the organisms has been a good help for biochemical approaches (Hamasaki et al., 1991). However, it is not useful for the analyses of the precise geometry of the bending pattern. Ciliary beating of ciliates is three dimensional (Mogami and Machemer, 1990) and difficult to reconstruct. In this study, I used sea urchin larvae to solve these problems. Cilia of sea urchin larvae are good materials for studying ciliary bend formation because they are sparse on the epithelium (one cilium per cell) and have highly planner configuration which can be captured in single focal plane of microscope (Baba, 1975; Baba and Mogami, 1987).

The development and growth of sea urchin larvae is accompanied by an increase in the complexity of their swimming behaviour. It has been suggested that this involves alterations in the mode of ciliary responses (Mogami *et al.* 1988, 1991). One of the most complicated movements established in the epaulette cilia of late stages of development is shown in Fig.I-2. Five states of movement have been reported in the epaulette cilia of the eight- armed pluteus: normal beating for forward (arm leading) locomotion, reversed beating for backward, intermediate beating in the course from

reversed to normal and two inactive states with upright and inclined positions. Ciliated cells acquire the ability to respond to neurotransmitters simultaneously with development of the nervous system in the pluteus stage (Nakajima, 1987). This coupling between nervous development and swimming behaviour indicate that ciliary movement may be regulated by nervous system.

It is demonstrated that Ca^{2+} act as a second messenger by modulating sliding- bending conversion. In ciliates, Ca^{2+} play a major role in inducing ciliary reversal as a second messenger (Naito and Kaneko, 1972; Mogami et al., 1990). It has also been demonstrated that cAMP regulates ciliary mechano- chemical cycle in both *in vitro* and *in vivo* (Hamasaki et al., 1991). In sea urchin larvae, Ca^{2+} decrease the swimming velocity in a concentration dependent manner (Degawa et al., 1986). It has been demonstrated that some neurotransmitters can change the behaviour probably by regulation via second messengers (Mogami et al., 1992).

In this paper, new aspects of neurotransmitter induced reactions of the cilia of sea urchin larvae were described and regulatory mechanisms of ciliary movements were discussed.

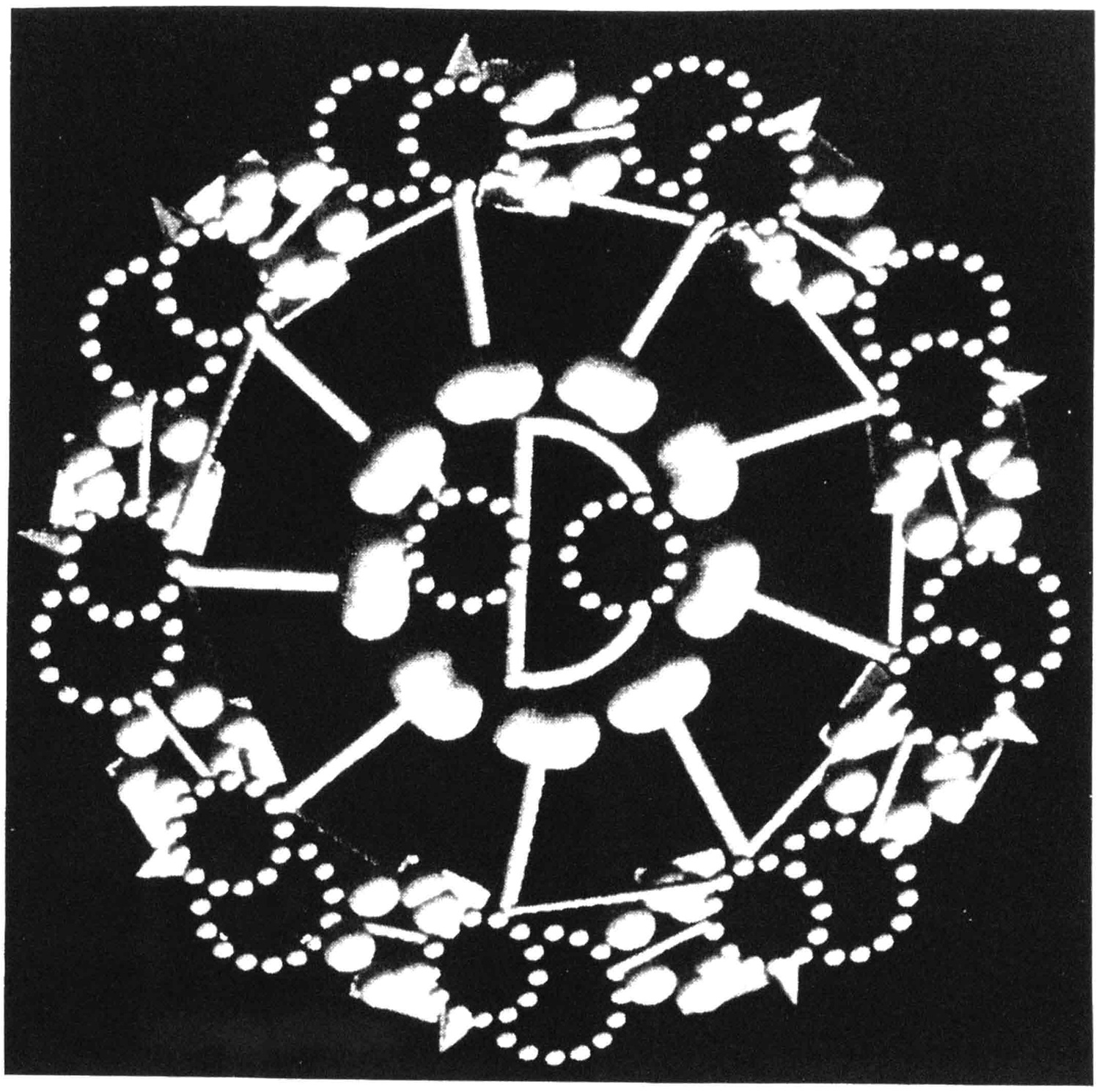

Fig. I–1. Computer model of cross–section of the axoneme with arms viewed from base to tip. (From Sugrue et al., 1991).

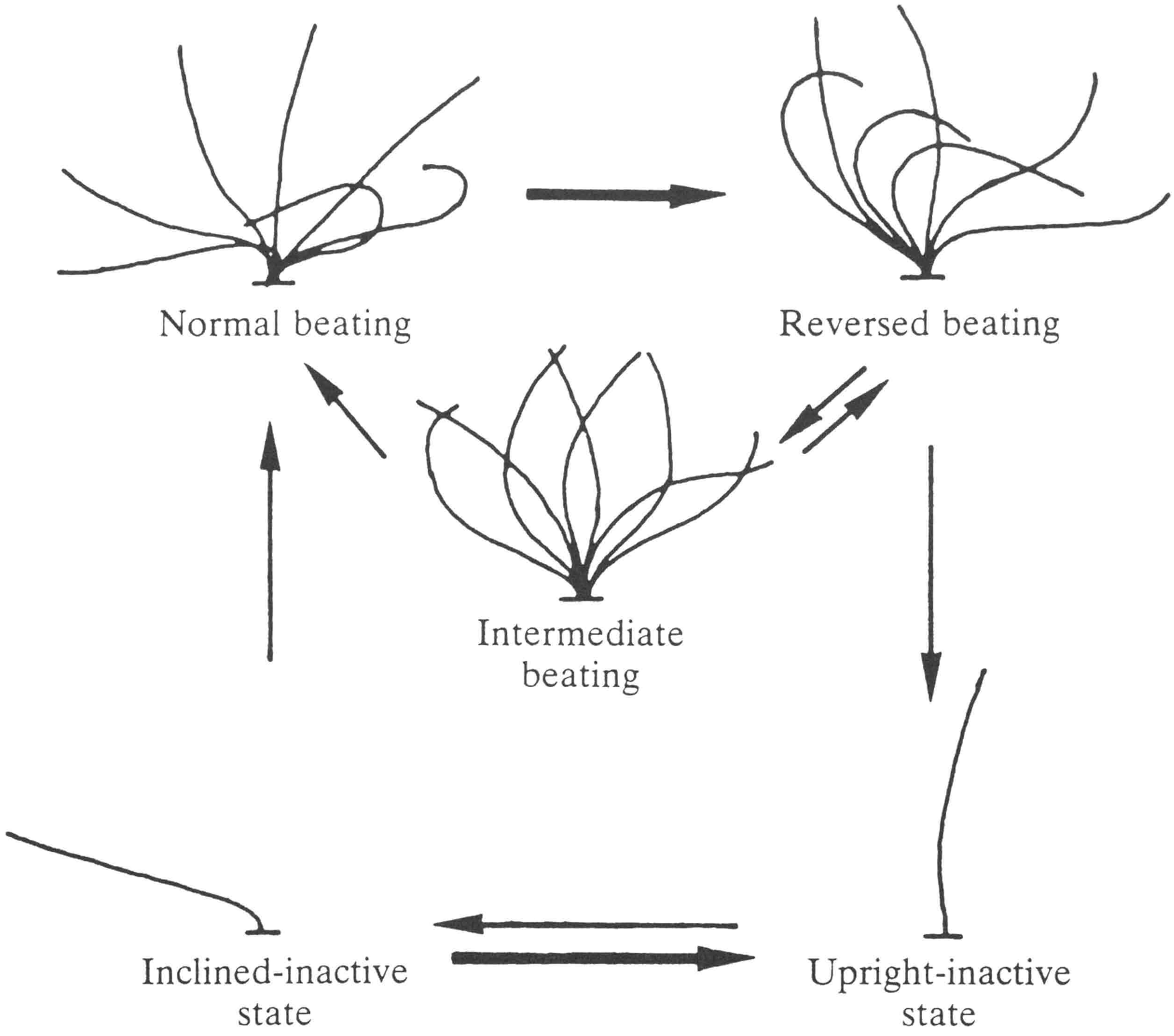

Fig. I–2. Five types of beating of epaulette cilia of eight–armed larva of *P. depressus*. Arrows indicate the direction of observed transitions. Thick arrows indicate faster steps (From Mogami et al., 1992).