Wing-beat mechanism of insect revealed by ultrafast X-ray movies

For aerodynamic reasons, small insects must beat their wings at high frequencies in order to fly. For example, the wing-beat frequencies of mosquitoes are around 500 Hz. It is very difficult to produce such fast oscillations by repeating ordinary contraction-relaxation cycles, each of which is elicited by a command from the motor nerve. Instead, these small insects have developed a special kind of flight muscles to drive their wings. These muscles are called "asynchronous flight muscles." These muscles are kept constantly activated during flight, and they undergo self-sustained oscillations. They are called asynchronous because their high-frequency oscillations are maintained by low-frequency nerve commands just to keep the muscles activated.

Although cross-striated as vertebrate skeletal muscles are, asynchronous flight muscles work with different principles. They do not move wings directly. They deform the thoracic exoskeleton that houses them, and the wings move up and down as a result of this deformation. There are left-right pairs of two antagonistic flight muscles, called dorsal longitudinal muscle (DLM) and dorso-ventral muscle (DVM). These pairs are arranged in a manner so that when one shortens, the other is stretched. Asynchronous flight muscles are equipped with a mechanism of stretch activation, a mechanism in which the muscle generates a large delayed force when stretched externally. When one of the antagonistic muscles shortens, it stretches the other. Then the stretched one generates a large force, and stretches back the other muscle. By repeating this process, the flight muscles can maintain self-sustained oscillations.

Thus, stretch activation is essential for the asynchronous action of flight muscles, but its molecular mechanism is still unclear despite decades of researches. Traditionally, isolated and demembranated flight muscle cells (skinned muscle fibers) have been used to study the molecular mechanism of stretch activation. Skinned fibers can be made to contract and relax by exchanging solutions, and generate stretch-activated force when stretched externally. They also give rise to high-quality diffraction patterns when irradiated with X-rays, and from these patterns we have extracted some clues to the behavior of constituent protein molecules when the fibers are stretch-activated. However, it remains unclear how accurately the observations made on skinned fibers reproduce the events undergoing in the flight muscles of live insects.

Here we have recorded ultrafast 2-dimensional X-ray diffraction movies from live bumblebees during wing-beat at an unprecedented rate of 5,000 frames/s, by using the high-flux **BL40XU** beamline [1]. The diffraction patterns were taken during "ethered flight," i.e., the bee was allowed to beat its wings while its body was fixed at a position by gluing its thorax to an aluminum tube. Bumblebees beat their wings at ~120 Hz (single wing-beat cycle, ~8 ms), i.e., ~40 frames/wing-beat, allowing detailed analysis. Two fast CMOS video cameras were used for data collection: One recorded the diffraction patterns, and the other recorded the wing-beat of the bee. The two cameras were synchronized by connecting them in a master-slave fashion, so that the X-ray frames were accurately correlated to the phase of wing-beat. The X-rays were irradiated at the point at which the two antagonistic flight muscles cross, so that their diffraction patterns were recorded at the same time.

Figure 1 shows one of the X-ray movie frames, created by summing data from 9 bees. A number of spot-like reflections are observed, and they originate from the near-crystalline arrangement of contractile proteins within muscle fibers. The reflections can be indexed by using Miller indices (*hkl*) as for other crystals. Patterns from both DLM and DVM are recorded on the same frame, and the reflections from these muscles can be distinguished because the axes of the two muscles make an angle of ~60°. In

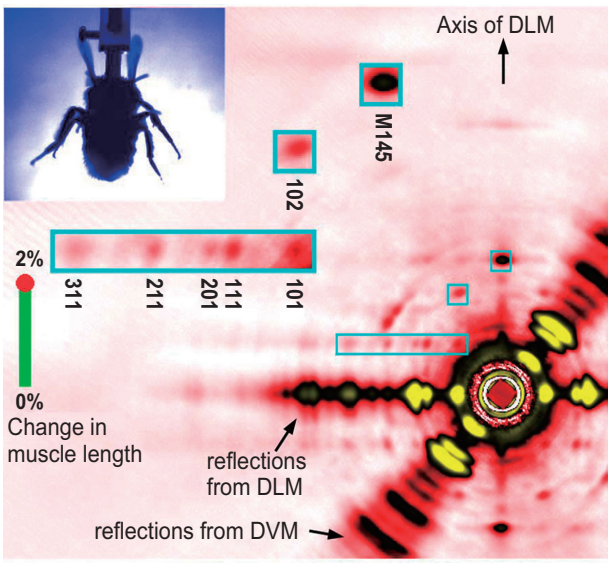

Fig. 1. One of the frames of X-ray diffraction movies. The pattern is re-oriented so that the fiber axis of DLM is vertical. [1]

the figure, the regions of reflections of interest are magnified in cyan boxes.

In the movie (provided as a supplemental material for the original paper), these reflections strengthen and weaken with the phase of wing-beat. By analyzing these reflections, the length change and force of muscle fibers can be estimated as well as the movement and structural change of constituent protein molecules. Figure 2 shows a part of the results of analyses. The top row for each muscle shows the estimate of change of muscle fiber length. The middle row shows the intensity changes of reflections reporting the strong attachment of myosin and actin, the two major contractile proteins in muscle. Their attachment and a subsequent structural change in myosin are believed to cause force generation. The bottom row is related to the most crucial finding in this study, and shows the intensities of two reflections, 111 and 201. A strengthening of 111 and a concomitant weakening of 201 occur in the stretch phase, a timing at which stretch activation occurs. Therefore these intensity changes may be related to a structural change that triggers stretch activation.

Figure 3 shows possible structural changes that account for the intensity changes of 111 and 201 reflections. Their reciprocal intensity changes can be caused by a mass movement from the blue area to the red area in the unit cell, and it is most readily explained by an azimuthal rotation of the tail region of myosin head (the blue object in Fig. 3. The part of myosin molecule that generates force). Then the mechanism of stretch activation may be explained as follows: The externally applied stretch causes distortion of myosin heads already attached to the actin filaments in the weak binding form. The distortion (azimuthal rotation) converts the myosin head from the weak binding form to the strong binding form, inducing force generation and enhancements of signals indicated in the middle row of Fig. 2.

A mechanism of distortion-induced enhancement of force has already been reported for vertebrate skeletal muscle [2], and therefore this may be a mechanism common to myosin in various types of muscles. If so, it can be said that insects have developed the mechanism of stretch activation by refining a pre-existing mechanism of myosin in ancestors common to both insects and vertebrates.

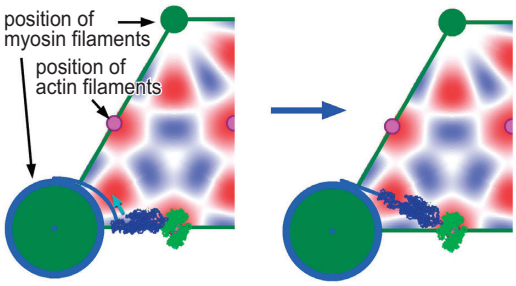

Fig. 3. Structural change within a unit cell that explains the observed intensity changes of 111 and 201 reflections. The 111 reflection is more intense than 201 in red areas, and vice versa in blue areas. [1]

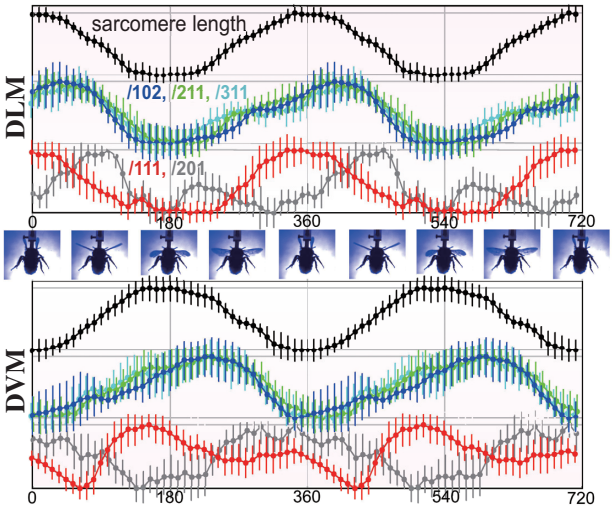

Fig. 2. Results of analyses of X-ray diffraction movies. Top rows, estimated changes of fiber lengths; middle rows, intensity changes of reflections that report myosin binding to actin; bottom rows, intensity changes of reflections considered to report the triggering events of stretch activation. The plots are made for 2 wing-beat cycles. [1]

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References

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