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Swimming against the tide

Toshio Yanagida rejects the conventional biophysical explanation of muscle contraction. No one doubts his technical genius, but could the debate he started ultimately hold back the field? David Cyranoski investigates.

Iconoclast, radical, technical wizard — these are just some of the descriptions that have been applied to Toshio Yanagida, host of last month's Frontiers in Molecular Motors Research symposium in Japan.

Yanagida's technological brilliance has been crucial in developing methods to study what drives muscle contraction. But for 15 years, he has been at odds with the majority of muscle biophysicists over the precise mechanisms involved.

Held on the island of Awaji, just across the water from Yanagida's laboratories in Osaka, last month's meeting was a lively affair. Both sides of the debate presented their latest results. And these served to underline the field's impressive achievements in manipulating and visualizing the individual protein molecules involved in muscle movement.

But from similar experiments, the participants drew very different conclusions. And this continuing trend for both camps to bolster their arguments without finding common ground leaves many in the field perplexed. The debate is where it was ten years ago, says Clive Bagshaw, a biophysicist at the University of Leicester. "It's like nothing has changed."

The argument centres on two proteins: myosin II and actin. Myosin II has two 'heads' joined by 'neck' regions to a coiled 'tail'. In muscle cells, the tails of myosin II molecules clump together to form 'thick filaments' with the necks and heads jutting out from the sides. Actin, on the other hand, forms helical 'thin filaments', which line up alongside their myosin counterparts. Muscles contract when the thick and thin filaments slide past one another. The puzzle for biophysicists is how the myosin II heads interact with the actin filaments to bring about this movement.

Head start

Most researchers back the lever-arm theory. In this, myosin II heads latch onto a nearby actin thin filament. By flipping in a lever-like motion, each head propels the actin in a 'power stroke' (see figure, opposite). The myosin head then lets go of the actin, the neck cocks back, and the head reattaches at a new point on the thin filament, starting the process again.

Muscle contraction is driven by adenosine triphosphate (ATP), the energy 'currency' of the cell, which binds to the myosin head. In the lever-arm model, a single cycle of actin attachment and power stroke for one myosin II head involves the hydrolysis of one ATP molecule. Because of this straightforward relationship between energy input and mechanical action, the lever-arm theory is also called the tight-coupling model.

Yanagida proposes that, for each ATP molecule consumed, a myosin II head moves several steps, bumping along the actin filament like a railway wagon rattling down a rickety track. But the details of his theory are frustratingly diffuse. "My opponents are always saying: 'give more interpretation of your results'," says Yanagida, "but I think it's better not to."

An input of ATP is still crucial, although its precise role in this 'loose-coupling' model is unclear. One possibility, says Yanagida, is that myosin somehow absorbs the energy from ATP hydrolysis, releasing this energy over a series of steps to drive its movement. But the role of ATP might simply be to cause a change in myosin's shape that allows movement to begin. In this case, much of the energy needed for myosin to move against actin would come from the Brownian motion of the molecules in the surrounding fluid. Brownian motion is the random movement of molecules, driven by thermal forces and characterized by collisions between the

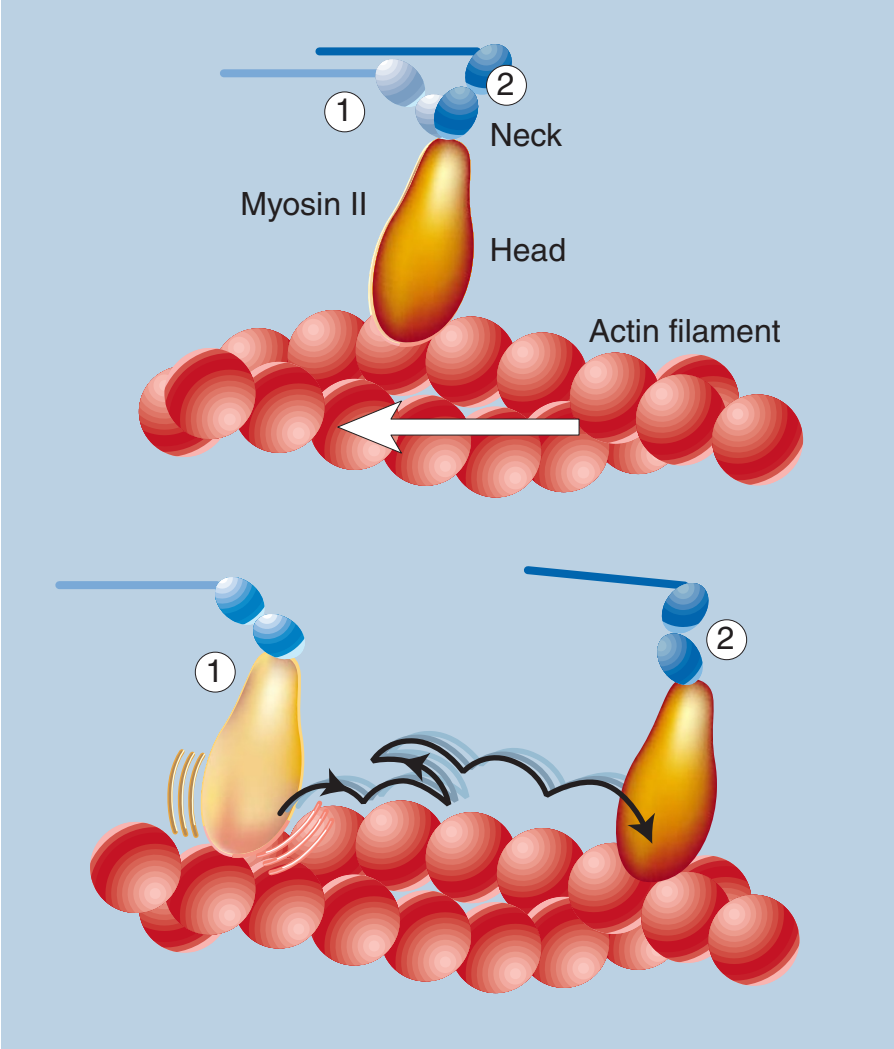
molecules. Yanagida believes that the structures of actin and myosin might bias this motion in one direction — although he does not have a full explanation of how this could work.

Seeds of doubt

Yanagida made his mark in 1984, when his group was the first to image a single actin filament in solution[1]. But it was in the following year that he sowed the seeds of the debate that continues to this day. By relating the 'sliding velocity' of an actin filament to the consumption of ATP, Yanagida estimated that a single molecule of ATP causes a myosin head to advance some 60 nanometres down an actin filament[2]. This posed a problem for the lever-arm theory, as the dimensions of the myosin head and neck mean that a single ATP molecule should cause a displacement of only around 6 nm. Japanese newspapers immediately hailed Yanagida as a scientific folk hero, claiming that he had overturned a popular 'Western' theory.

Yanagida was working with his mentor Fumio Oosawa at Osaka University, even though Oosawa could only employ him as a technician. After Oosawa retired, the university took the unusual step in 1988 of promoting Yanagida straight to full professor. He has since enjoyed high levels of government funding. His current grant, for his Single Molecule Processes project, is worth more than US$9.3 million over five years to 2002.

With this generous financial support, Yanagida has assembled a team of 15 scientists in a renovated warehouse. Most have a strong physics or engineering background, and have built their own equipment more or less from scratch. Their technical accomplishments, it is widely agreed by their peers, represent a *tour de force*.



Flipping neck: the motion of actin and myosin II filaments relative to each other causes muscle contraction. In the lever-arm theory (top), myosin heads repeatedly attach to the actin fibre and then flip forward, propelling the actin along. But according to Yanagida's loose-coupling theory (bottom), the myosin runs along the actin fibre like a railway wagon on a rickety track.

Image is everything

In the 1980s, biophysicists realized that solving the mysteries of muscle contraction would require better technology. They wanted to trap and manipulate individual protein molecules tagged with fluorescent markers. And they needed sophisticated microscopes to watch these proteins interact.

Yanagida helped to overcome many of these hurdles, building the necessary tools in his lab — and, in doing so, spurred others to higher levels of technological sophistication. "He has pushed the envelope," says Ron Vale, a biophysicist at the University of California at San Francisco.

In 1995, for instance, Yanagida's group revealed the first images of a single myosin II molecule binding to an ATP molecule in an aqueous solution[3]. Imaging fluorescently tagged proteins in solution using conventional microscopes is difficult because the laser that makes the molecules visible also causes scattering and luminescence throughout the solution. This problem was solved by a protégé of Yanagida's, Takashi Funatsu. He refined a technique called total internal reflection fluorescence so that he could limit to 150 nm the depth to which the laser's light penetrated the solution. This set up an 'evanescent' light field, which illuminated the target molecules but reduced the background luminescence more than 2,000-fold.

Other experiments had a more direct bearing on Yanagida's loose-coupling theory. In 1998, Yanagida and his colleagues reported that they had simultaneously observed the binding of ATP and recorded the displacement of a single myosin molecule along an actin filament[4]. The experiment combined Funatsu's evanescent field with another key tool — 'optical tweezers'. These were first applied to muscle biophysics by researchers working in the lab of James Spudich at Stanford University in California[5]. By fixing microscopic beads to either end of an actin filament, the filament can be held taut by directing laser beams — the tweezers — at the beads. From his experiments, Yanagida concluded that the motion of a myosin head down an actin filament induced by one ATP molecule did not occur immediately. He observed a delay of several tenths of a second between the release of adenosine diphosphate, the product of ATP hydrolysis, and the myosin's motion. This delay is incompatible with the tight-coupling model.

Last year, Yanagida and his colleagues refined their estimate of 60 nm for the myosin displacement induced by a single ATP molecule to between 11 and 30 nm (ref. 6). This revision resulted from measurements taken during experiments in which individual myosin II heads were attached to glass microneedles. By measuring the deflection of the needles and their stiffness, the researchers recorded both the displacement of the myosin heads and whether or not they were attached to the actin filament.

They concluded that a single molecule of ATP causes a myosin head to move down the filament in a series of distinct steps, each about 5 nm long. Yanagida cannot rule out

that the myosin head detaches from the filament momentarily during these steps, but he argues that if this happens it is too fast for the myosin to hydrolyse another ATP molecule, and that the net effect is one of loosely coupled movement.

Necks on the block

The problem is that most of the other groups working in the field, using similar imaging approaches to those used by Yanagida, have consistently recorded results that fit the lever-arm theory. They remain convinced that there is a tight coupling between ATP and myosin displacement, and measure this displacement at less than 10 nm (refs 5,7,8). Further evidence for their case comes from structural studies, which indicate that myosin can bend its neck as proposed by the lever-arm model[9–12].

At the Osaka symposium, both sides presented fresh results. A key discussion focused on myosin's neck region. According to the lever-arm theory, changing the length of the molecule's neck should alter myosin's power stroke, and hence the displacement induced by a single ATP molecule. But Yanagida predicts that neck length will make little difference.

David Warshaw of the University of Vermont described experiments published last month[13] in which his group halved the length of the neck of myosin II. Sure enough, this decreased by 40% the average displacement induced by one ATP molecule — and increasing the neck length had the opposite effect. But Yanagida's colleagues described unpublished experiments in which they removed the neck of myosin II altogether, and found no difference in displacement.

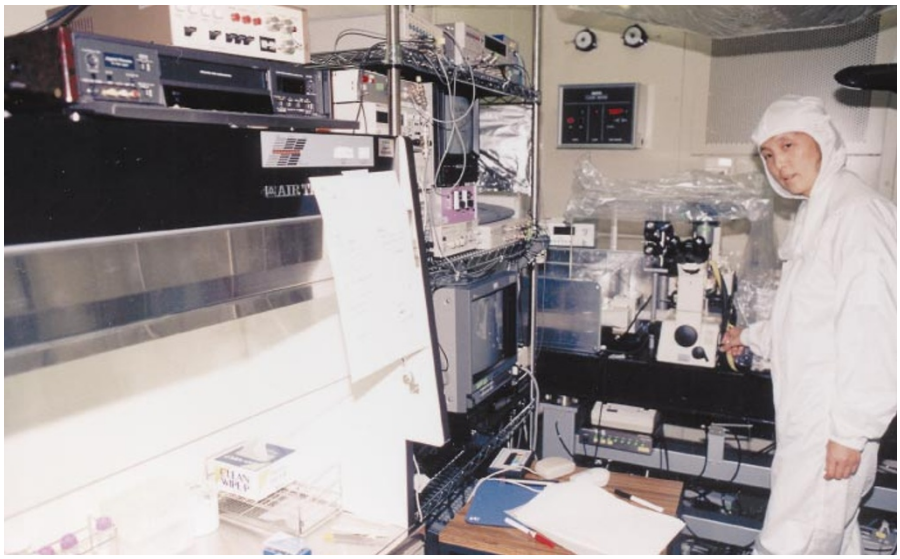
Every which way but loose?

Reconciling conflicting results in this field is extremely difficult — especially given the huge investment of time and money needed to create the experimental set-ups. Although everyone in the field acknowledges these constraints, some of Yanagida's competitors feel that his approach makes resolving the debate almost impossible.

"The problem with the loose-coupled thermal ratchet model is that it is difficult to devise experiments to specifically exclude it," says Justin Molloy of the University of York. "The tightly coupled lever-arm idea is simple, predictive and inherently testable because of its more restrictive nature."

Yanagida's glass microneedle experiment, for instance, is controversial. Is the myosin head really positioned on the glass needle as described in the paper? Might there be two myosin heads attached, the fluorescent one, plus one that has lost its fluorescence? If so, this might help explain the multiple steps that Yanagida sees.

But anyone wanting to repeat the experiment faces a formidable challenge. The microneedles were produced by Kazuo Kitamura, who says it took him six months of practice before he perfected their manufacture. "No one wants to repeat this experiment in Japan — not even in our own group," says Yoshiharu Ishii, group leader for Yanagida's Single Molecule Processes project.



Field project: Takashi Funatsu's experimental set-up allows muscle proteins to be seen in action.

Yanagida admits that his loose-coupled model can appear a little vague. "But just because I can't explain the whole system doesn't mean my data are wrong," he insists.

In explaining the continuing difference of opinions, Yanagida hints at cultural rifts. In Eastern thought, "it is necessary to pile experimental fact upon fact before asserting anything to be true", he says. This makes Yanagida and his supporters — most of whom are Japanese — comfortable with the absence of a simple, complete model.

But to most muscle biophysicists, this is no excuse for Yanagida's failure to provide a better description of his theory. And they find it difficult to accept his cheerful admission that he rejects the tight-coupling model on intuitive and aesthetic grounds. "It's so boring," he says.

Yanagida invokes his original training as a semiconductor physicist. Transistors, he explains, are fast and simple. "Biological molecules are not that simple," he says, holding his elbow with one hand and pumping it from side to side in imitation of a lever. "They are too soft, too flexible." He also questions the significance of the lever-arm camp's structural studies. "They cannot tell us about function," he argues.

Confusion reigns

Given the difficulty of repeating Yanagida's experiments, many of his competitors complain that they are left to grapple with unassailable data shrouded in a ghost-like theory. One researcher at the Osaka meeting likened him to the US Second World War general George Patton, who gained a reputation for making relentless advances, leaving his colleagues to sort out the mess left behind in his wake. "Sometimes I wonder if he's really looking for answers or just out to cause trouble," observes another biophysicist.

Despite the exasperation felt by many in the field, the arguments do not seem to have become tainted with personal animosity. Yanagida is amiable and generally well-liked. And at the Osaka meeting, he maintained a light-hearted playfulness even while disagreeing strongly with some of his peers. Nevertheless, his steely determination becomes clear when asked why he continues to swim against the tide of scientific opinion. "Because I'm right," he responds.

Most biophysicists agree that Yanagida has so far been a force for good, his charisma and technical skills helping draw money into the field. But some researchers fear that the field is becoming mired in an ultimately unproductive debate.

Perhaps the best summing up of the current state of play came in the Osaka symposium's closing speech. "I came here confused about actin and myosin," said Nobel laureate Andrew Huxley of the University of Cambridge. "Now, I am still confused, but at a higher level." ■

David Cyranoski is *Nature*'s Asian-Pacific correspondent.

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