Molecular motors as an auto-oscillator

Shin'ichi Ishiwata,^{1,2,3} Yuta Shimamoto,⁴ and Madoka Suzuki^{1,3}

¹Department of Physics, Faculty of Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan

²Advanced Research Institute for Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan

³Waseda Bioscience Research Institute in Singapore (WABIOS), 11 Biopolis Way, No. 05-01/02 Helios, Singapore 138667, Singapore

⁴Laboratory of Chemistry and Cell Biology, Rockefeller University, New York, NY 10065, USA

(Received 24 March 2010; published online 15 April 2010)

The organization of biomotile systems possesses structural and functional hierarchy, building up from single molecules via protein assemblies and cells further up to an organ. A typical example is the hierarchy of cardiac muscle, on the top of which is the heart. The heartbeat is supported by the rhythmic contraction of the muscle cells that is controlled by the Ca^{2+} oscillation triggered by periodic electrical excitation of pacemaker cells. Thus, it is usually believed that the heartbeat is governed by the control system based on a sequential one-way chain with the electrical/chemical information transfer from the upper to the lower level of hierarchy. On the other hand, it has been known for many years that the contractile system of muscle, i.e., skinned muscle fibers and myofibrils, itself possesses the auto-oscillatory properties even in the constant chemical environment. A recent paper [Plaça, *et al.* (2009), *Phys. Rev. Lett.* 103, 158102] demonstrated the auto-oscillatory movement/tension development in an *in vitro* motility assay composed of a single actin filament and randomly distributed myosin II molecules, suggesting that the auto-oscillatory properties are inherent to the contractile proteins. Here we discuss how the molecular motors may acquire the higher-ordered auto-oscillatory properties while stepping up the staircase of hierarchy. [DOI: 10.2976/1.3390455]

CORRESPONDENCE

Shin'ichi Ishiwata:
ishiwata@waseda.jp

Various kinds of auto-oscillation phenomena exist in biomotile systems: heartbeat is a typical example. A pacemaker cell produces periodic electrical impulses that spread across the whole heart, controlling the heartbeat by orchestrating the contraction of cardiac cells. Another example comes from hair cells, which are the mechanosensors present in the inner ear of vertebrates, playing a key function in hearing. This system displays mechanical oscillations coupled with opening/closing of transduction channels such that mechanical stimuli can be amplified (Martin *et al.*, 2003). These oscillation phenomena observed at the cellular level are usually regulated by changes in the concentration of small signal molecules such as Ca^{2+} ions. In this sense, mechanical oscillations are tightly coupled with chemical oscillations and the mechanical apparatus, therefore, seemingly functions as a passive element.

Electrical impulses are also involved in the regulation of auto-oscillation of indirect flight muscle in insects; however, the oscillation frequency is not directly coupled to the frequency of electrical impulses or chemical signals. This oscillation, powered by actin-myosin system, is controlled by the resonance with the mechanical framework of the thorax, characteristic to each insect (Pringle, 1978). Another interesting phenomenon is the beating of cilia and flagella in protozoa, which occurs through the relative sliding of microtubule-dynein systems in the axoneme structure in the absence of any external control. Moreover, such oscillation phenomena driven by motors working on cytoskeletal filaments are not limited to the so-called “motile” systems; namely, the mitotic spindle, and cell nucleus also show auto-oscillation at some stage of cell division (Pecreaux *et al.* 2006; Vogel *et al.*, 2009), involving the dy-

Figure 1. Analysis of the auto-oscillatory force development observed in the A-band motility assay system and a skeletal myofibril. (A) Schematic illustration of the A-band motility assay (nanomuscle) system (left) and a myofibril system (right). (B–D) Oscillatory myofilament movement and tension development observed in the A-band motility assay system (B, outside the A-band; C, inside the A-band) and a myofibril (D, a typical pattern of SPOC). (Top) The time course of the displacement of an actin filament (B and C) or sarcomere length (the upper part of D); the corresponding changes in tension in the A-band motility system are shown on the right vertical axis (B and C); the tension developed by a myofibril is shown at the middle of D. (Bottom of B–D) Spectral densities of the data shown in the top row. The data in B and C were taken from a part of those presented in [Suzuki et al. \(2005\)](#). For the SPOC data, see [Shimamoto et al. \(2007\)](#).

namic rearrangement of cytoskeletal components in the cell. In all these systems, the communication between force-generating motors may be playing an essential role in controlling the auto-oscillatory phenomena.

SPONTANEOUS OSCILLATORY CONTRACTION (SPOC) IN MYOFIBRILS

A myofibril of striated (skeletal and cardiac) muscle would be one of the most obvious examples of mechanical oscillations emerging from the assemblies of contractile proteins. The myofibrils are composed of a series connection of sarcomeres and represent a membraneless structural and functional unit of striated muscle. The stable auto-oscillation in individual sarcomeres occurs under the controlled conditions of intermediate activation between full activation and relaxation. This phenomenon is termed spontaneous oscillatory contraction (SPOC, [Ishiwata and Yasuda, 1993](#)). Importantly, SPOC is not a chemical oscillation, as it occurs at constant concentrations of chemical constituents but is induced by the collective action of myosin motors.

SPOC was observed in both cardiac muscle fibers/myofibrils ([Fabiato and Fabiato, 1978](#); [Linke et al., 1993](#); [Fukuda et al., 1996](#); [Fukuda and Ishiwata 1999](#); [Fujita and](#)

[Ishiwata, 1998](#); [Sasaki et al., 2005, 2006](#)), skeletal muscle fibers ([Stephenson and Williams, 1982](#); [Shimizu et al., 1992](#)), and skeletal myofibrils ([Okamura and Ishiwata, 1988](#), [Anazawa et al., 1992](#); [Yasuda et al., 1996](#); [Ishiwata et al., 2007](#); [Shimamoto et al., 2007, 2008, 2009](#)) under various conditions including partial Ca^{2+} activation conditions. The three-dimensional phase diagram showing the occurrence of three states, i.e., contraction, relaxation and SPOC, in relation to various concentrations of free Ca^{2+} (pCa), inorganic phosphate (Pi), and Mg-ADP in the presence of a fixed concentration of Mg-ATP was constructed ([Ishiwata and Yasuda, 1993](#); [Ishiwata et al., 2007](#)). The SPOC region in the three-dimensional phase diagram is much larger in the case of cardiac muscle than skeletal muscle, implying that cardiac muscle is better tuned for the occurrence of SPOC.

The characteristics of SPOC are: (1) the length of each sarcomere in a myofibril (a muscle fiber) auto-oscillates with a saw-tooth waveform, consisting of slow shortening and quick lengthening [Fig. 1(D)] and (2) this sarcomere length oscillation propagates along a myofibril (SPOC wave). Furthermore, the period of sarcomere length oscillation and the velocity of SPOC wave both correlate well with the resting heartbeat of each animal species, i.e., rat, rabbit, dog, pig,

and cow (Sasaki *et al.*, 2005, 2006). Thus, the SPOC properties may play important roles in heartbeat. In relation to the physiological function of the SPOC properties, it is to be noted that the traveling wave similar to the SPOC wave transiently occurs upon the transition from contraction to relaxation in cardiac muscle (Stehle *et al.*, 2002, 2006).

Several theories, from general to specific, have been proposed to explain the auto-oscillatory properties of SPOC. For example, the theory of Jülicher and Prost (1995) (J&P theory) was a general one, as it describes randomly distributed myosin molecules forming cross-bridges with an actin filament on which asymmetric interaction potentials are linearly and periodically distributed. A specific theory was developed, for example, by Smith and Stephenson (2009), which incorporated various realistic parameters based on the mechanical and structural characteristics of sarcomere. Furthermore, we recently constructed a simple theory to explain almost all the dynamic properties of SPOC and to represent a phase diagram consisting of contraction, SPOC, and relaxation states with a small set of parameters (K Sato, M Ohtaki, Y Shimamoto, and S Ishiwata, manuscript to be submitted). Although the mechanism of auto-oscillation has been proposed experimentally and theoretically, the minimal system required for the oscillation had yet to be revealed.

RANDOM ASSEMBLY OF MYOSIN MOTORS AS AN AUTO-OSCILLATOR

In a recent work, Plaçais *et al.* (2009) reported auto-oscillatory movement in an *in vitro* motility assay system, which consists of a bead-tailed single actin filament interacting with several myosin motors. The bead was trapped by optical tweezers, which functioned as an elastic spring. In this experimental system the myosin molecules were randomly distributed on the two-dimensional glass surface, which is analogous to that assumed in the J&P theory. The authors showed that their minimal actomyosin system coupled with an elastic load could produce auto-oscillations and the theoretical approach based on the J&P theory successfully reproduced the oscillatory phenomena. Interestingly, they observed two characteristically different types of oscillations in the movement of single actin filaments (10–100 nm in amplitude with an average period of several seconds), namely, the saw-tooth type and the bimodal type. The former looked similar to SPOC in muscle and the latter resembled the oscillation in hair cells, suggesting that different types of oscillation in different organs may emerge from a simple switching of mechanical parameters in the same minimal contractile system.

In their experiments, the number of the involved motor molecules was estimated to be 10–100 in total, of which a few tens were working simultaneously at any given moment. They found that the absolute force, which reflects the number of force-generating myosin molecules at any given time, did not correlate with the oscillation parameters assumed in

the J&P theory. Instead, the trap stiffness showed a good correlation with the parameters. According to their results, the oscillatory characteristics are presumably determined by the stiffness of the system, whereas the number of the involved motors affects the noise level.

An important feature of the experimental system used by Plaçais *et al.* (2009) is that an upward force is exerted on the myosin molecules as they pull the actin filament and the bead away from the trap center (Fig. 1 in Plaçais *et al.*, 2009). Kishino and Yanagida (1988) avoided this geometrical complexity by incorporating pedestals in their assay system (in this work a glass microneedle functioned as an elastic spring). It remains to be seen whether this difference in the experimental geometries significantly affects the obtained results.

However, even though the more detailed theoretical and experimental analysis is still required, especially on the molecular level, we now know that a minimal system composed of a small number of myosin II motors and a single actin filament can generate spontaneous oscillation with a characteristic frequency. The coupling of this system with other chemomechanical oscillators as external controllers will be one of the interesting topics toward the understanding of the remarkable features of self-regulation that the real biological systems possess.

HIERARCHICAL ORGANIZATION OF OSCILLATION PATTERN

Another interesting question concerning the function of a nonprocessive molecular motor myosin II as an auto-oscillator is: How does the spatiotemporal organization of oscillation, i.e., molecular synchronization, occur as the ordering of the geometrical arrangement of actomyosin molecules is increased? This led us to examine the time course of tension development (equivalent to the sliding movement of a single actin filament) in the A-band motility assay system [Fig. 1(A) left, we call it a “nanomuscle,” Suzuki *et al.* (2005)] in a similar way as used by Plaçais *et al.* (2009). The A-band motility assay system has been developed aimed at bridging the gap between an *in vitro* motility assay system and a myofibril system (Molloy, 2005).

Figure 1 (B, top, and C, top) shows the time courses of the movement of single bead-tailed actin filaments outside and inside the A-band, respectively. In both geometries the waveforms appeared to be saw-toothed. However, they often disappeared within a few cycles by fluctuations, which is similar to the observations by Plaçais *et al.* (2009). In these experiments, the time-averaged overlap length of the actin filament in the A-band is calculated to be 95 nm for Fig. 1(B) and 86 nm for Fig. 1(C), corresponding to the number of the available myosin molecules being 6.8 and 9.2, respectively. Figure 1 strongly suggests that the organization of the oscillation pattern improves as the interaction space becomes more regular [from the outside (B) to the inside (C)]

Figure 2. Schematic illustration showing structural and functional hierarchy in cardiac muscle system. (i) A single-molecule level, (ii) randomly distributed myosin molecules with a single actin filament, (iii) regularly assembled myosin molecules (a bundle of thick filaments, i.e., an A-band) with a single actin filament, (iv) sarcomeres and myofibrils, (v) muscle cells, and (vi) the heart. At each level of hierarchy, time courses of changes in force development, $[Ca^{2+}]$ and/or electrical impulse are also schematically depicted.

of the thick filament lattice in an isolated A-band], approaching a typical SPOC pattern observed in a single myofibril [Fig. 1(D)]. The ordering of the oscillation pattern seems to be achieved at the level of hierarchy where a nanoscopic filament lattice composed of the thick (myosin) and thin (actin) filaments appears.

CONCLUSION

As shown schematically in Fig. 2, the organization of the muscle system possesses structural and functional hierarchy, i.e., builds up from (i) a single-molecule level (a pair of a single myosin motor and a single actin filament), where the force development (that is, the movement of a myofilament) is stochastic; (ii) a random assembly of actomyosin molecules, where the characteristics as an auto-oscillator appear; (iii) a sarcomere composed of a regular crystalline array of myofilaments, where a periodic auto-oscillation termed SPOC occurs; (iv) a myofibril (a serial array of sarcomeres)

and a muscle fiber (a parallel bundle of myofibrils) without cell membrane (skinned fibers; we call this “a contractile system of muscle”), where the SPOC wave occurs; and (v) a muscle cell (the contractile system covered with cell membrane), where the auto-oscillatory properties of the contractile system of muscle are regulated by changes in the Ca^{2+} concentration triggered by electrical impulse. The heartbeat occurs at the level of organ, which is located at the highest level of functional hierarchy of the heart (vi). There exist auto-oscillatory properties characteristic of each level of hierarchy. We now know that the molecular motors are intrinsically an auto-oscillator, which implicates that they may be the basis for the beautifully organized functions observed at the higher level of hierarchy.

ACKNOWLEDGMENTS

All authors equally contributed to the preparation of manuscript. The authors thank Dr. S.V. Mikhailenko for his critical reading of the manuscript. The present research was supported by the Grants-in-Aid for Specially Promoted Research, Scientific Research (A) and the “Academic Frontier” Project (to S.I.) and Young Scientist (B) (to M.S.) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. This research was also supported by the Asia-Africa Science and Technology Strategic Cooperation Promotion Program, Special Coordination Funds for Promoting Science and Technology from the MEXT of Japan (to S.I.). Y.S. is a JSPS postdoctoral fellow for research abroad.

REFERENCES

- Anazawa, T, Yasuda, K, and Ishiwata, S (1992). “Spontaneous oscillation of tension and sarcomere length in skeletal myofibrils. Microscopic measurement and analysis.” *Biophys. J.* **61**, 1099–1108.
- Fabiato, A, and Fabiato, F (1978). “Myofilament-generated tension oscillations during partial calcium activation and activation dependence of the sarcomere length-tension relation of skinned cardiac cells.” *J. Gen. Physiol.* **72**, 667–699.
- Fujita, H, and Ishiwata, S (1998). “Spontaneous oscillatory contraction without regulatory proteins in actin filament-reconstituted fibers.” *Biophys. J.* **75**, 1439–1445.
- Fukuda, N, Fujita, H, Fujita, T, and Ishiwata, S (1996). “Spontaneous tension oscillation in skinned bovine cardiac muscle.” *Pflügers Arch. Gesamte Physiol. Menschen Tiere* **433**, 1–8.
- Fukuda, N, and Ishiwata, S (1999). “Effects of pH on spontaneous tension oscillation in skinned bovine cardiac muscle.” *Pflügers Arch. Gesamte Physiol. Menschen Tiere* **438**, 125–132.
- Ishiwata, S, Shimamoto, Y, Suzuki, M, and Sasaki, D (2007). “Regulation of muscle contraction by Ca^{2+} and ADP: focusing on the auto-oscillation (SPOC).” *Adv. Exp. Med. Biol.* **592**, 341–358.
- Ishiwata, S, and Yasuda, K (1993). “Mechanochemical coupling in spontaneous oscillatory contraction of muscle.” *Phase Transitions* **45**, 105–136.
- Jülicher, F, and Prost, J (1995). “Cooperative molecular motors.” *Phys. Rev. Lett.* **75**, 2618–2621.
- Kishino, A, and Yanagida, T (1988). “Force measurements by micromanipulation of a single actin filament by glass needles.” *Nature (London)* **334**, 74–76.
- Linke, WA, Bartoo, ML, and Pollack, GH (1993). “Spontaneous sarcomeric oscillations at intermediate activation levels in single isolated cardiac myofibrils.” *Circ. Res.* **73**, 724–734.
- Martin, P, Bozovic, D, Choe, Y, and Hudspeth, AJ (2003). “Spontaneous

- oscillation by hair bundles of the bullfrog's sacculus." *J. Neurosci.* **23**, 4533–4548.
- Molloy, JE (2005). "Muscle contraction: actin filaments enter the fray." *Biophys. J.* **89**, 1–2.
- Okamura, N, and Ishiwata, S (1988). "Spontaneous oscillatory contraction of sarcomeres in skeletal myofibrils." *J. Muscle Res. Cell Motil.* **9**, 111–119.
- Pecreaux, J, Röper, JC, Kruse, K, Jülicher, F, Hyman, AA, Grill, SW, and Howard, J (2006). "Spindle oscillations during asymmetric cell division require a threshold number of active cortical force generators." *Curr. Biol.* **16**, 2111–2122.
- Plačais, P-Y, Balland, M, Guérin, J-F, Joanny, J-F, and Martin, P (2009). "Spontaneous oscillations of a minimum actomyosin system under elastic loading." *Phys. Rev. Lett.* **103**, 158102.
- Pringle, JW (1978). "Stretch activation of muscle: function and mechanism." *Proc. R. Soc. London, Ser. B* **201**, 107–130.
- Sasaki, D, Fujita, H, Fukuda, N, Kurihara, S, and Ishiwata, S (2005). "Auto-oscillations of skinned myocardium correlating with heartbeat." *J. Muscle Res. Cell Motil.* **26**, 93–101.
- Sasaki, D, Fukuda, N, and Ishiwata, S (2006). "Myocardial sarcomeres spontaneously oscillate with the period of heartbeat under physiological conditions." *Biochem. Biophys. Res. Commun.* **343**, 1146–1152.
- Shimamoto, Y, Kono, F, Suzuki, M, and Ishiwata, S (2007). "Nonlinear force-length relationship in the ADP-induced contraction of skeletal myofibrils." *Biophys. J.* **93**, 4330–4341.
- Shimamoto, Y, Suzuki, M, and Ishiwata, S (2008). "Length-dependent activation and auto-oscillation in skeletal myofibrils at partial activation by Ca^{2+} ." *Biochem. Biophys. Res. Commun.*, **366**, 233–238.
- Shimamoto, Y, Suzuki, M, Mikhailenko, SV, Yasuda, K, and Ishiwata, S (2009). "Inter-sarcomere coordination in muscle revealed through individual sarcomere response to quick stretch." *Proc. Natl. Acad. Sci. U.S.A.* **106**, 11954–11959.
- Shimizu, H, Fujita, T, and Ishiwata, S (1992). "Regulation of tension development by MgADP and Pi without Ca^{2+} . Role in spontaneous tension oscillation of skeletal muscle." *Biophys. J.* **61**, 1087–1098.
- Smith, D, and Stephenson, DG (2009). "The mechanism of spontaneous oscillatory contractions in skeletal muscle." *Biophys. J.* **96**, 3682–3691.
- Stehle, R, Kruger, M, and Pfitzer, G (2002). "Force kinetics and individual sarcomere dynamics in cardiac myofibrils after rapid Ca^{2+} changes." *Biophys. J.* **83**, 2152–2161.
- Stehle, R, Solzin, J, Iorga, B, Gomez, D, Blaudeck, N, and Pfitzer, G (2006). "Mechanical properties of sarcomeres during cardiac myofibrillar relaxation: stretch-induced cross-bridge detachment contributes to early diastolic filling." *J. Muscle Res. Cell Motil.* **27**, 423–434.
- Stephenson, DG, and Williams, DA (1982). "Effects of sarcomere length on the force-pCa relation in fast- and slow-twitch skinned muscle fibers from the rat." *J. Physiol. (London)* **333**, 637–653.
- Suzuki, M, Fujita, H, and Ishiwata, S (2005). "A new muscle contractile system composed of a thick filament lattice and a single actin filament." *Biophys. J.* **89**, 321–328.
- Vogel, SK, Pavin, N, Maghelli, N, Jülicher, F, and Tolic-Nerrellykke, IM (2009). "Self-organization of dynein motors generates meiotic nuclear oscillations." *PLoS Biol.* **7**, e1000087.
- Yasuda, K, Shindo, Y, and Ishiwata, S (1996). "Synchronous behavior of spontaneous oscillations of sarcomeres in skeletal myofibrils under isotonic conditions." *Biophys. J.* **70**, 1823–1829.