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Review

Processive cytoskeletal motors studied with single-molecule fluorescence techniques

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ABSTRACT

Processive cytoskeletal motors from the myosin, kinesin, and dynein families walk on actin filaments and microtubules to drive cellular transport and organization in eukaryotic cells. These remarkable molecular machines are able to take hundreds of successive steps at speeds of up to several microns per second, allowing them to effectively move vesicles and organelles throughout the cytoplasm. Here, we focus on single-molecule fluorescence techniques and discuss their wide-ranging applications to the field of cytoskeletal motor research. We cover both traditional fluorescence and sub-diffraction imaging of motors, providing examples of how fluorescence data can be used to measure biophysical parameters of motors such as coordination, stepping mechanism, gating, and processivity. We also outline some remaining challenges in the field and suggest future directions.

1. Introduction

A eukaryotic cell depends on a multitude of molecular motors, protein machines that convert chemical energy into mechanical work, to actively maintain the spatial organization and material flux required for the cell's survival. Molecular motors span several protein superfamilies, exhibiting remarkable diversity in structure and function to fulfill their wide variety of biological roles. The motors of the cytoskeleton are divided into three protein superfamilies. Kinesin and dynein motors bind to and translocate along the microtubule network, whereas myosin motors function on actin (Fig. 1A). These motors share several principal characteristics: they all use adenosine triphosphate (ATP) as the source of chemical energy and perform mechanical work by walking along their respective track. The study of motors through biochemical methods is complicated by the fact that many of the fundamental properties of their motility cannot be readily measured in bulk assays. One such property is the motor's velocity, which determines how rapidly it can deliver cargo to its destination. Another is processivity, a measurement of how many successive steps a motor can take before dissociating from its track, which is critically important for understanding how teams of motors work together to power long-distance transport while avoiding gridlock and overcrowding. For a more detailed understanding of the motor's mechanism, it is invaluable to know its stepping pattern – the manner in which the heads move with respect to one another as the motor walks down its track. These properties are all readily amenable to study with single-molecule fluorescence techniques.

2. Diffraction-limited single motor imaging

Motors that function in muscle contraction (myosin II) and ciliary beating (inner and outer arm dyneins) work in large groups to generate force on macroscopic scales. While these motors can be studied collectively with filament gliding assays [1], individual

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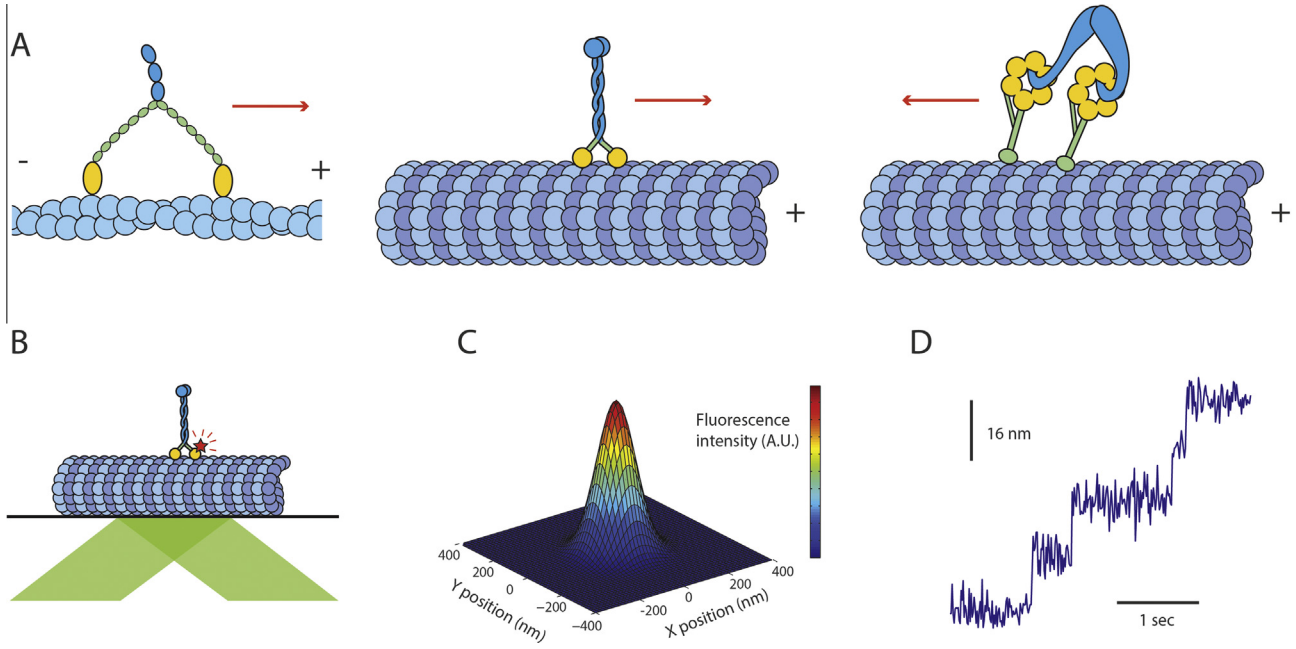

Fig. 1. Processive cytoskeletal motors and fundamentals of sub-diffraction TIRF imaging. (A) Three classes of processive cytoskeletal motors: myosin V (left) walks towards the plus end of actin filaments, kinesin-1 (center) walks towards the plus end of microtubules, and cytoplasmic dynein (right), walks towards the minus end of microtubules. (B) Schematic depiction of a TIRF motility assay (not to scale). A fluorescently labeled motor (kinesin-1 is shown) walks on a surface-immobilized track. The fluorophore is excited by the evanescent field of a collimated laser beam (green) reflecting off the glass/water interface. (C) The point-spread function (PSF) of a single fluorophore is well approximated by a 2-dimensional Gaussian. By collecting a sufficiently large number of photons per frame, the center of the PSF can be localized with nanometer precision. (D) By localizing a fluorophore attached to a walking motor protein over many consecutive frames and plotting the position of its center as a function of time, one can obtain stepping traces similar to the simulated trace shown here. Such traces can then be processed with a step-finding algorithm and used to extract biophysical parameters such as dwell times, stepping rates, and step sizes.

motors need not be processive and their motility may not be immediately apparent on a single-molecule level. However, it was soon discovered that many other cytoskeletal motors transport cargos in small teams or alone [2,3], a function requiring the molecules to be able to take many successive steps without diffusing away from the track. In order to achieve processive motion, a molecular motor must remain tethered to the track throughout its entire mechanochemical cycle, a requirement that potentially explains why the vast majority of processive motors discovered to date possess two or more track binding sites.

The first direct confirmation of motor processivity was achieved by imaging individual kinesin molecules walking along microtubules [4], and was soon followed by similar observations on myosin [3] and dynein [5]. Single-motor motility assays are performed under total internal reflection fluorescence (TIRF) [6] illumination, in which the evanescent field of a laser beam reflected off the water/glass interface excites fluorescently tagged motors moving along surface-immobilized tracks (Fig. 1B). The intensity of the evanescent field falls off exponentially with distance from the coverslip, limiting the depth of the excitation region to a few hundred nanometers and greatly reducing background fluorescence from the bulk solution. Observing the motors directly in real time allows for measurement of a number of fundamental properties. Kinesin-1 was shown to travel on average 600 nm before dissociating from the track, demonstrating that a typical run consisted of ~100 mechanical cycles [4] assuming the previously measured 8 nm step size [7]. Repeating the experiment with kinesin constructs lacking their dimerization domain showed that kinesin-1 requires both heads to remain processive. It has furthermore been shown that Unc104 [8] and myosin VI [9] motors transition from diffusional to directional processive motion upon dimerization at high concentrations. The requirement for dimerization for processive motility was also demonstrated in yeast cytoplasmic dynein by designing monomers with chemically inducible dimerization domains [5].

3. Regulation of motors

A cell employs regulatory mechanisms to control the attachment of motors to cargos, to modulate their velocity or force production depending on the specific task they're performing, or to prevent them from undergoing futile cycles of ATP hydrolysis when not engaged with the track [10]. Such mechanisms can be grouped into two general categories: autoinhibition and inhibition by small molecules or regulatory proteins. Motility experiments on kinesin-1 mutants with the tail domain either truncated or made less flexible at a prominent hinge showed that both mutants moved 2 to 3-fold faster than wild-type kinesin and exhibited greatly enhanced processivity. This points towards an autoinhibition mechanism wherein kinesin's tail acts as a repressor of the motor domain in the absence of bound cargo [11]. Crystallographic work later showed that this inhibition occurs via a tail-mediated crosslinking of the two motor domains, preventing the separation of the two heads required for neck linker undocking [12]. Similar autoinhibitory mechanisms appear to be present in kinesin-2 [13], kinesin-3 [14], and myosin V [15–17] motors.

For cytoplasmic dynein, several distinct regulatory proteins were identified. Lis1 impacts dynein motility on a single-molecule level [18], effectively anchoring dynein to its track. Interestingly, this mechanism does not prevent futile cycles of ATP hydrolysis, suggesting that dynein may also have an autoinhibitory mechanism yet to be discovered. Lis1-based anchoring potentially configures dynein for low-speed, high-force cellular tasks such as anchoring spindle microtubules during mitosis. Another dynein regulator, She1, diffuses along microtubules until it encounters a walking dynein. She1 binds and pauses the motor, prolonging its attachment to the microtubule [19]. A small-molecule inhibitor, monastrol, was used to target homotetrameric kinesin Eg5, which slides apart microtubules and contributes to the assembly of the mitotic spindle [20]. The effect of monastrol in Eg5 motility was