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Single-molecule studies of nucleic acid motors

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Nucleic acid motors comprise a variety of structurally, mechanistically and functionally very different enzymes. These motor proteins have in common the ability to directionally move DNA or RNA, or to move along DNA or RNA using a chemical energy source such as ATP. Recently, it became possible to study the action of a single motor on single DNA or RNA molecules in real time; this has provided unprecedented insight into the behavior and mechanism of these motors. As a result, the past few years have witnessed an enormous increase in such single-molecule studies of a variety of different motor systems. Particular highlights have included the investigation of the sequence-dependent behavior and helical tracking of motors, and the attainment of the ultimate (i.e. single base pair) resolution, which enables the detection of individual single base motor steps.

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Introduction

Nucleic acid motors are ubiquitous in all fundamental cellular processes that involve DNA or RNA, for example, transcription, replication, translation, packaging and maintenance of chromosome structure, restriction, DNA repair and bacterial conjugation. Most motor proteins use ATP or other nucleotide triphosphates (NTPs) to move along nucleic acids. Very often, such a simple translocation process is accompanied by another nucleic acid remodeling function. For example, RNA polymerase moves along the DNA, reads out its sequence and transcribes it into RNA. Helicases unzip or unwind duplex DNA to allow other proteins to access the DNA strands. To understand the function and mechanism of these enzymes, it would be greatly beneficial to observe a single enzyme moving along DNA or RNA at atomic resolution in real time. This, however, is currently impossible. On one hand, crystal structure data provide the necessary spatial resolution, but crystallographic methods only afford snapshots of individual states of proteins, that is, the dynamics of the process is lacking. On the other hand, classical biochemical analyses provide dynamic information, but average over large molecular ensembles. Single-molecule techniques allow the observation of the dynamic action of a single motor protein on a DNA or RNA molecule with a resolution that is now better than the 0.34 nm of single base pair steps [1••]. Single-molecule experiments also provide unique access to parameters such as the forces produced by motors and offer an excellent complement to classical techniques to obtain an understanding of motor function at the atomic level.

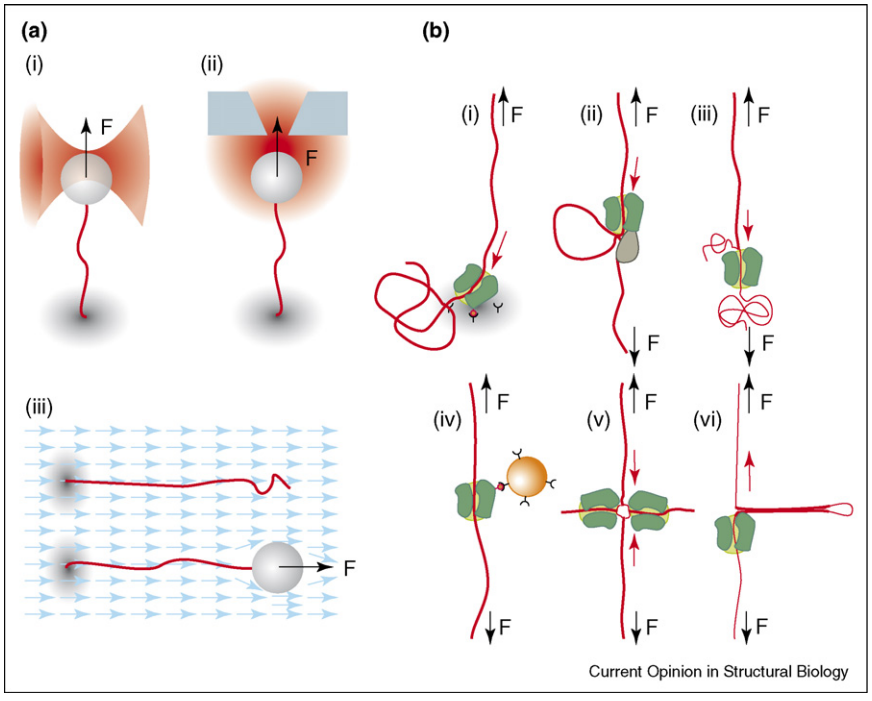
This review summarizes the achievements of single-molecule methods in the field of nucleic acid motors during the past two years. In particular, the direct observation of single motor steps, the study of DNA helix tracking, the investigation of the sequence-dependent behavior of motor movement and the unraveling of the mechanochemistry of single motors will be discussed. Furthermore, a perspective will be given on future challenges and possible developments for the field.

How to measure translocation of a single motor

Most assays currently used to observe the action of nucleic acid motors start with the stretching of DNA or RNA molecules using an external force that is applied by a hydrodynamic flow or optical or magnetic tweezers (see Figure 1a). Motor activity is then measured either as a change in the DNA or RNA length or directly from the position of a labeled motor on the stretched DNA or RNA (Figure 1b). Other single-molecule methods, such as fluorescence resonance energy transfer (FRET; see [2]), have also been applied to motor proteins; however, to date, they have not been used to directly measure translocation. Optical tweezers, the preferred method of choice so far, provide currently the highest spatial and temporal resolution, whereas magnetic tweezers conveniently allow supercoils to be introduced by applying torque to the DNA and enable measurements to be made at extremely low constant forces (as low as ~0.01 pN). Hydrodynamic flow, on the other hand, is technically the least complicated method.

To transform motor movement along DNA into a change in the measured length of the stretched nucleic acid structure or the position of a marker bound to the motor, several strategies have been followed depending on the

Figure 1



Single-molecule methods for measuring translocation of nucleic acid motors. **(a)** Most methods rely on stretched DNA or RNA. Stretching can be achieved using: **(i)** a laser trap, which can pull on a molecule that is attached between a surface and a bead; **(ii)** a magnetic field, which exerts force on a superparamagnetic bead; or **(iii)** a hydrodynamic flow, which acts on the DNA or RNA molecule itself (top) or on a bead attached to the molecule (bottom). **(b)** Motor movement on a stretched nucleic acid molecule can be detected in different ways. **(i)** Immobilizing the motor and detecting the shortening or lengthening of the DNA or RNA molecule. **(ii)** Motors that translocate DNA or RNA into loops directly shorten the molecule; this can be detected. **(iii)** For motors that convert ssDNA to dsDNA or dsDNA to ssDNA (such as DNA polymerases or helicases), a change in the molecule length can be detected, because ssDNA and dsDNA have different extension depending on the stretching force. **(iv)** Direct observation of a translocating motor on stretched DNA or RNA. Visualization of the motor is achieved by labeling it with (fluorescent) beads or fluorescent antibodies or by simple visual detection of protein aggregates. **(v)** Motors such as RuvAB, which specifically move a homologous four-way junction, can be detected when DNA is pumped from two branches of a given orientation into the perpendicularly oriented branches. **(vi)** Unzipping of a hairpin by a helicase. Unzipping each base pair increases the length of the ssDNA or ssRNA part by two bases. Red arrows symbolize the direction of DNA movement, black arrows the direction of the applied force, F.

actual motor system (Figure 1b): (i) immobilization of the motor at the surface of a glass slide or a bead held by a micropipette or an optical trap [1••,3,4••,5•,6,7]; (ii) monitoring the change in length as a result of DNA loop formation during translocation [8•,9–11,12•,13–16]; (iii) using the different force-dependent extensions of dsDNA and ssDNA [17••]; (iv) direct observation of motor movement along a stretched DNA molecule by detecting protein aggregates [13,14] or fluorescent beads or antibodies that are attached to the motor [14,18,19]; (v) using branch migration of a homologous four-way junction [20,21]; and (vi) measuring the unzipping of a hairpin by a helicase [22•].

The use of one of these methods can powerfully extract relevant motor properties at the single-molecule level. For example, in the assay shown in Figure 1b(iv), one can track a particle attached to the motor. In principle, this is now possible at an ~1 nm resolution [23]. Following the particle over time directly yields the velocity and typical processivity (translocation length) of a single motor on DNA. Typical values are given in Table 1. A wide range of parameters can be noted, in line with the varying needs of the different cellular functions of the motors (Box 1).

Resolving single motor steps

The ultimate goal in detecting motor movement is to resolve individual motor steps or substeps, as they provide interesting insight into motor function and mechanism. Whereas the steps of cytoskeletal motors, most famously the 8.2 nm steps of kinesin on microtubules [24], were resolved long ago, similar experiments on nucleic acid motors remained challenging, as their steps are on the order of a single or a few base pairs. Recently, however, the 0.3 nm single base pair detection limit was broken using sophisticated ultrastable optical tweezers [1••] and it became possible to detect the single base pair stepping motion of RNA polymerase on DNA (Figure 2). This now opens the way to answering mechanistic questions regarding the motor stepping motion and resolving

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