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MOLECULAR MOTORS: DNA takes control

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Abstract

Polarized arrays of microtubules can be assembled and disassembled using motor proteins that are programmed by DNA strands.

Motor proteins play an essential role in a variety of self-assembly processes in cells. They are, for example, involved in the internal organization of the cytoplasm, consuming ATP as fuel to transport subcellular materials along cytoskeletal filaments. Networked or crosslinked motors can also drive the self-assembly of organized filament structures (such as the mitotic spindle of dividing cells) by manipulating the positions and orientations of their filament tracks¹. These highly dynamic and non-equilibrium self-assembly processes are, however, difficult to emulate in the laboratory. Writing in *Nature Nanotechnology*, Andrew Turberfield and colleagues at the University of Oxford and Warwick Medical School now show that complexes made from DNA and multiple motor proteins can be used to assemble and disassemble filament structures in a cell-free environment².

Organized complexes of multiple molecular motors have been created before by using either proteins or DNA as a molecular scaffold^{3,4}. These systems were primarily developed to examine the mechanisms governing collective motorforce production and transport behaviours, which are important in many transport and trafficking processes in cells. Furthermore, DNA self-assembly techniques such as DNA origami⁵ have been used to create larger and more complex multiple motor ensembles. In particular, DNA origami has been used to connect different types of motor, which could then compete in a molecular ‘tug of war’⁶. Building on such methods, Turberfield and colleagues use DNA polymers to template the assembly of multiple motors. However, the DNA–motor hybrids they create also have a unique feature: partially duplexed DNA domains that can be dehybridized via an isothermal chemical reaction called DNA strand displacement^{7–9}.

In the DNA-strand-displacement reaction, a DNA strand is designed, or programmed, to displace another strand in a double-stranded DNA molecule, and the researchers use this reaction to regulate the coupling of the motors (Fig. 1a). As a result, the DNA–motor complexes can be used to control the organization of networks of microtubule filaments (hollow tube-like polymers made of α - and β -tubulin protein dimers). Specifically, DNA-coupled motors, which contain two kinesin molecules, can crosslink two microtubule

filaments and then orient the filaments as they walk towards the plus end of the microtubules (Fig. 1b, step 1); similar capabilities have been reported before with motor complexes formed using streptavidin–biotin chemistries^{10, 11}. At a sufficiently high motor-complex concentration, the process leads to the formation of aster-like microtubule patterns (Fig. 1b, step 2), similar to the starburst-like clusters of polarized microtubules that are found at opposite ends of the bipolar mitotic spindle.

Although these aster structures are kinetically stable, Turberfield and colleagues show that they can be disassembled by using the strand-displacement mechanism to break the DNA link between motors in complexes that are situated at the centre of the aster (Fig. 1b, steps 3–4). Simply adding single-stranded DNA oligonucleotides to the solution can trigger the strand-displacement reaction and aster ‘melting’, but the team shows that the structures can be disassembled more efficiently by using a modified hybrid complex, which contains only a single kinesin motor protein, to actively transport the required DNA ‘disassembly signal’ strand to the centre of the aster.

Turberfield and colleagues also demonstrate that the ordered microtubule structures can be used as trackways for cargo transport controlled by DNA signals. DNA shuttles with single kinesin motors attached can scavenge fluorescently labelled DNA cargo from solution and concentrate it at the centre of an aster. Separate shuttles can then transport DNA ‘release signals’ that displace the cargo by strand displacement and release it back into solution.

The ability to build and tune the organization of complex cytoskeletal structures in a cell-free environment could have a number of scientific and technological implications. The capabilities could help provide insight into the basic mechanisms that underlie the dynamics of cellular structures, such as mitotic spindles of dividing cells, or could be employed in different motor-protein-based transport applications. For example, patterned microtubule arrays and motor transport machinery have previously been integrated into biologically inspired optical devices that recapitulate the colour-switching mechanisms in pigment cells called melanocytes, which are found in certain species of fish¹². The dynamic DNA–motor hybrids may offer new ways of regulating the actions of motors, and the organization of filament networks in these types of device.

In the future, alternative control mechanisms and additional functionalities could also be developed for the hybrid complexes themselves. For example, optical switches⁶ could regulate the coupling between motors, or techniques could be employed to tune the velocity and directionality of the individual motors¹³. Such developments may ultimately lead to the design of new classes of adaptive bio-inspired materials that cannot be readily made using traditional, thermodynamically driven self-assembly techniques.

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Figure 1.

Controlled assembly and disassembly of aster-like microtubule networks using hybrid complexes made of DNA and motor proteins. **a**, Motors within a hybrid complex can be controllably disconnected using a strand-displacement reaction. The reaction occurs between a partially hybridized oligonucleotide (orange) that is incorporated into a DNA-motor complex containing two kinesin motors (each motor is shown as a green rectangle connected to two white circles) and a second DNA-motor complex that contains a single kinesin motor and a single-stranded DNA disassembly signal. **b**, DNA-motor complexes that contain two motor proteins can crosslink and orient microtubules as they walk towards the plus ends of the microtubules (step 1). This leads to a focusing of the microtubules at their plus ends and the formation of aster-like microtubule patterns (step 2). Following aster formation, the disassembly signal is added to the solution in the absence of ATP (which acts as fuel for the motors). After the initial binding of the disassembly signals to the aster microtubules, ATP is added, which leads to the transport of the disassembly signal towards the centre of the aster (step 3). The subsequent decoupling of the two-motor complexes (via the strand-displacement reaction) drives the disassembly of the polar microtubule network (step 4).