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13. ABSTRACT (Maximum 200 words)

Methods have been developed for depositing patterned distributions of any antibody molecule on a SiO_2 wafer with high spatial definition and signal-to-noise ratio (Mooney et al., PNAS, 1996). This method is now being used to attach microtubule organizing centers (centrosomes) in patterned arrays to develop organized microtubule geometries of known polarity. In a parallel investigation, antibodies bound to glass have been used to attach chromosomes isolated from mammalian cells to a substrate for light microscopy. Stable microtubule fragments have then been bound to the chromosomes, elongated with rhodamine labeled tubulin, and their dynamics studied both by direct inspection and during intervention by a laser trap, holding a bead affixed to the chromosome distal end of the labile microtubule. Both chromosomes and centrosomes are examples of microtubule organizing centers with mechanical properties that can contribute to future work on engineered nanomachines.

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J.R. McIntosh and C.T. Rogers, Univ. Colorado, March 31, 1997

FINAL REPORT

In Vitro Microtubule Growth for Producing Engineered Nanotransport Machines

TWO PRONGS TO OUR APPROACH

1. Isolate functional cell parts that can bind microtubules in vitro
2. Devise a photolithography method to pattern functional proteins on SiO_2.

ACCOMPLISHMENTS

1. Chromosomes bound to glass by antibodies.
   Labeled, stable fragments of microtubules bound to chromosomes.
   Microtubule fragments work as seeds for polymerization of tubulin subunits.
   Elongating microtubules add subunits at the kinetochores as they polymerize.
   Kinetochore-bound microtubules will also depolymerize, retaining their attachments.
   Results demonstrate that glass-bound chromosomes retain microtubule binding activity.
   Design and build an optical trap, based on a neodinium-YAG laser, that can exert >20pn on glass microspheres of 1μm diameter.
   Use trap to attach microspheres coated with avidin to biotinylated segments of microtubule attached to chromosomes and assess strength of the attachment.
   Use trap to measure forces generated by microtubules as they polymerize.

2. Glass derivatized with a monolayer of n-octadecyl trimethoxysilane (OTS) will bind a monolayer of bovine serum albumin (BSA).
   The OTS monolayer can be patterned by UV photolithography.
   A layer of biotinylated BSA will bind a layer of Streptavidin.
   A layer of Streptavidin will bind a layer of biotinylated antibody.
   This layer of antibody is still functional to bind its antigen.
   This multilayered sandwich shows the pattern of the original lithogram.
   Employ adsorbed antibodies to attach centrosomes to glass and begin a study of ordered microtubule assembly in vitro.

SIGNIFICANCE

Tools are now in hand to organize the position and orientation of microtubules in vitro. These polymers can bind motor enzymes, the ultimate in micro-miniaturization of mechanochemical devices. We are on our way to constructing a nanomachine of unprecedented significance.

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INSTITUTION: University of Colorado

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OBJECTIVE: To investigate the utility of biological objects that can initiate and/or bind microtubules for the control of microtubule organization in vitro. These objects will then be ordered by a novel method for binding active proteins to surfaces patterned by microlithography and used to assemble ordered arrays of polymers for the construction of a nanomachine.

APPROACH: Chromosomes and centrosomes are isolated from mammalian cells to serve as sites for microtubule organization in vitro. These objects are bound to glass and their interactions with microtubules characterized by light microscopy and by experiments with laser tweezers. In a parallel line of investigation, surfaces are prepared that can bind antibodies and then patterned by photolithography. These lines of investigation will then be put together to construct ordered arrays of microtubules of defined polarity which can in turn interact with motor enzymes to make nanomachines.

ACCOMPLISHMENTS We have devised a protocol by which chromosomes are bound by antibodies to glass coverslips while they retain their ability to bind microtubules. Stable fragments of microtubules labeled with rhodamine can bind to the kinetochore region of the chromosomes. The activity of the kinetochore is displayed by its ability to keep a hold on one end of these microtubules as they elongate by the addition of tubulin at the kinetochore, pushing the labeled seed away from the chromosome. The microtubules retain their dynamic behavior, undergoing occasional "catastrophes," in which they change to a state of rapid shortening, and "rescues," in which they change back to the state that favors growth. The elongation of a kinetochore-associated microtubule is at the same rate as a free microtubule in the same preparation; the rate of rapid shortening is, however, reduced by a factor of five.

We have purchased a neodinium-YAG laser and used it to construct an optical trap that will hold transparent, refractile spheres strongly enought that >20pN are required to pull a 1μm glass sphere from the trap. We have calibrated the spring constants of the trap at several levels of laser power and demonstrated the linearity of the relationship between displacement of the sphere from the optic axis and the force applied. This tool has been used to show that 8pN is not sufficient to remove a dynamic microtubule from the kinetochore to which it is bound, but 20pN is. We have also shown that 2pN sufficient to stop the polymerization-dependent movement of a microtubule away from a kinetochore. These results are now being assembled for publication.

We have also devised a method for creating patterns of functional antibodies, using photolithography of n-octadecyl trimethoxysilane (OTS) monolayers on silicon dioxide surfaces, followed by adsorption of biotinylated bovine serum albumin (BSA) and streptavidin. Under proper solutions conditions, OTS-treated SiO_2 adsorbs a monolayer of BSA, while untreated SiO_2 binds less than 2% of a monolayer. Ultra violet photolithographic patterning of OTS prepares

a surface for high contrast patterned adsorption of biotinylated BSA, which can be followed by the patterned chemisoption of streptavidin and thence any biotinylated protein. We have demonstrated that functional antibodies can be patterned by this process (Mooney et al., 1996).

These methods are now being used to bind centrosomes isolated from CHO cells to glass, where they are fully active in initiating the growth of microtubules in vitro. Centrosomes will now be pattered, using the above photolithography methods to produce ordered microtubule arrays for organized nanotransport.

SIGNIFICANCE: The motor enzymes that interact with protein polymers, like microtubules and actin microfilaments, represent the ultimate in mechnical microminiaturization: a single macromolecule can transduce fuel into mechanical work, resulting in a motion whose direction is defined by the orientation of a biopolymer. This "biotechnology" might be useable in the construction of nanodevices of unprecedented sophistication. Our approach utilizes cell biological objects to perform a series of tasks essential for device construction: the initiation of polymer growth, the establishment of fiber polarity, and the generation of the mechanical forces. We then use a novel lithography setup to create well defined patterns of antibodies and thus of virtually any macromolecule of biological significance.

PUBLICATIONS AND ABSTRACTS (last 12 months):

Hunt, A.J. and McIntosh (1996) Direct observation of polymerization-linked movement of chromosome-bound microtubules. Biophys. J. 70:A44(abs).

Mooney, J.F., C.T. Rogers, A.J. Hunt, and J.R. McIntosh (1996) A general technique for patterning of functional proteins with photolithorography of silane monolayers. Biophys. J. 70:A300(abst).

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Hunt, A.J. and McIntosh (1997) The dynamic behavior of individual microtubules associated with chromosomes in vitro. to be submitted to Molecular Biology of the Cell.

Signed, J. Richard McIntosh

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