

probes on the basis of a different emission or absorption characteristics.

The fragment of interest is manipulated to an extended condition, preferably corresponding to a selected stretching force, as above, and the particles are attached to the chamber surface, as by optical adherence. The extended filament is now examined to determine the distance between fluorescent-labeled restriction-site probes, typically by measuring the distances between probe sites seen in the video camera images. As shown in FIG. 10A, the fragment contains six rare restriction sequences s_1-s_6 which define five restriction segments f_1-f_6 , with the relative measured lengths shown in the figure. The distances between each of the restriction sites and known sequence A are also recorded.

A higher resolution restriction map can now be made by introducing a fluorescence probe for a more frequent restriction site, under hybridization conditions discussed above. The more frequent sites typically have average spacings of about 50-100 kbases. FIG. 10B shows an enlargement of segment f_5 , with probes specific to the more frequent restriction site being bound at sites $s_{5.1}$ to $s_{5.5}$ between previously identified sites s_5 and s_6 . The seven restriction sites define six subsegments $f_{5.1}$ to $f_{5.6}$ in segment f_5 , as indicated. The lengths of these subsegments are determined as above.

A more detailed restriction map may be constructed in this manner by addition of probes specific to other restriction sites. The identified segments may be isolated at any stage by restriction site digestion and fractionation by electrophoresis, according to standard procedures. For example, following the two-probe analysis above, genomic fragments may be digested to completion with the rare cutter restriction enzyme, e.g., NotI, and subfragments having the expected segment size, e.g., of fragment f_5 , then isolated from the gel. These subfragments may be further digested to completion with the second, more frequent restriction enzyme, and the smaller subfragments again fractionated by gel electrophoresis. Smaller subfragments, e.g., $f_{5.4}$, are identified on the gel by their known size and isolated. These isolated fragments can now be cloned for sequencing, and/or expression, or further analyzed by the mapping method just described.

For high resolution distance measurements, the filament can be suitably prepared for electron microscopy or force field microscopy.

A variety of sequence-specific binding molecules, such as restriction enzymes, enhancers, repressors, transcriptional or translational initiation or termination factors, histones, and ribosomes may be substituted for nucleic acid probes, for localization of binding sites on an extended filament. These DNA-binding agents can be fluorescently labeled by known methods of derivatizing proteins with fluorescent reporters.

In a second general embodiment, the extended filament serves as a substrate for nucleic-acid specific enzymes or ribosomes, for real-time measurements of the rate and/or mechanism of interaction of enzymes or ribosomes with extended DNA. For example, in applying the method to the study of ribosome binding to mRNA, filaments of mRNA are prepared by known methods, coupled at opposite ends to particles, and extended by the optical trap manipulation methods described above. With the mRNA in an extended condition, in vitro translation components are added to the liquid film. Among the determinations which can be

made in the method are (i) the time sequence in which the ribosomes become attached to the mRNA filament; (ii) the rate of movement along the filament; and (iii) the fate of the ribosomes in the presence of various translation inhibitors, i.e., whether the inhibitor stops ribosome movement along the strand or causes the ribosomes to detach from the mRNA.

The method may similarly be used to study the mechanisms and kinetics of attachment and movement of RNA or DNA polymerases, reverse transcriptases, reverse topoisomerases (in a pair of crossed, extended filaments) and repair enzyme along an extended DNA filament, employing fluorescently-labeled enzymes.

Although the invention has been described with respect to particular embodiments and methods, it will be clear to those skilled in the art that various changes and modifications can be made without departing from the invention.

It is claimed:

1. A method of preparing a polymer filament for microscopic examination in an extended condition, comprising

coupling one end of the filament to a particle in the size range of about 10 nm to 10 μ m,

suspending the filament and attached particle in a fluid film in a chamber,

securing the other end of the filament in the chamber, capturing the particle in an optical trap produced by directing a beam of divergent, coherent light through a collimating lens and directing the resulting collimated beam through a high-numerical aperture objective lens, where the collimating lens is positioned to (a) shift the angle by which the collimated beam produced by directing the divergent beam through the collimating lens is directed against the objective lens, thereby to shift the position of said optical trap produced by directing the collimated beam through the objective lens, and (b) maintain the position of the collimated beam substantially fixed in the plane of the objective lens, so that the beam fills the lens at any beam angle and the light intensity of the trap is substantially independent of position, and

moving the source of the divergent light, to produce a corresponding movement of the optical trap, until the filament is in an extended condition.

2. The method of claim 1, wherein said filament is a nucleic acid filament with a 5'-end phosphate group at said one filament end, said particle has surface amine groups, and said coupling steps includes reacting the filament with the particle in the presence of a carbodiimide coupling reagent, to link said one filament end to the particle through a phosphoamidate bond.

3. The method of claim 1, wherein the particle has a size between about 0.1 and 1 μ m.

4. The method of claim 1, which further includes attaching the particle to the chamber when the filament is in an extended condition.

5. The method of claim 4, wherein said attaching includes positioning the particle against a surface of said chamber, and holding the particle at a substantially stationary position in the optical trap for a period sufficient to adhere the particle to the chamber surface.

6. The method of claim 4, which further comprises adjusting the power of the divergent beam source, to produce a trapping force equal to a selected stretching force of the filament, manipulating the particle to a position at which the particle can just escape from the