Introduction:

The study of motor proteins has become a major focus in cell and molecular biology. Motor proteins are very interesting because they do what no man-made engines do: they directly convert the chemical energy into mechanical energy without using any electrical energy or heat as an intermediate. Motor proteins are enzymes that convert chemical energy into motion. Chemical energy is obtained from the hydrolysis of ATP and the motion is generated by the conformational changes depending on the bound nucleotide such as myosin, kinesin and dynein. Motor proteins play an important role in muscle contraction, cell migration, chromosome segregation, morphogenesis, beating of sperms and cilia, transport of intracellular cargoes etc. They have also tremendous applications in nanotechnology. In the present lecture we have mainly focused on the actin based motor protein i.e. myosin.

Common properties of motor proteins:

- They move along the filaments.
- They can bind to specific filament types.
- They hydrolyze ATP.

Types of Motor proteins:

Generally there are two types of motor proteins-

1. **Actin based motor protein**: Myosin is an actin based motor protein. It moves along the actin filaments.

2. **Tubulin based motor protein**: Tubulin is the building block of microtubules. Kinesin and dynein are tubulin based motor proteins. They move along the microtubule. Kinesin motor moves towards plus (+) end of microtubule (away from centrosome) whereas dynein moves towards minus (-) end of the microtubules (towards centrosome).

There are another type of motor proteins called **Nucleic acid based motor proteins**. They move along a DNA and produce force. DNA and RNA polymerases are nucleic acid based motor proteins.
Evolution of motor proteins:

Nucleotide switch
Motor Precursor (Kyosin)

Kinesins
Kinesin Subfamilies

Myosins
Myosin Subfamilies

G proteins

Examples of Kinesin Subfamilies

Conventional (Dimer)
Direction: Plus End
Processivity: Yes
Biological Activities: Membrane transport

Ncd (Dimer)
Direction: Minus End
Processivity: No
Biological Activities: Mitotic Spindle Function

Examples of Myosin Subfamilies

Myosin I (Monomer)
Direction: Barbed End
Processivity: No
Biological Activities: Cell motility/membrane functions

Myosin II (Dimer)
Direction: Barbed End
Processivity: No
Biological Activities: Muscle Contraction/Cytokinesis

Myosin V (Dimer)
Direction: Barbed End
Processivity: Yes
Biological Activities: Membrane/mRNA transport

Myosin VI (Dimer)
Direction: Pointed End
Processivity: Unknown
Biological Activities: Membrane Transport

Now before going to the details of the motor proteins we have to discuss about the structure of them.

Microtubules are hollow cylinders composed of α, β tubulin heteromers and actin is a helical polymer of 8 nm diameter. Actin is more flexible than microtubule. Both actin and microtubule are polar and the polarity is due to the fact that the individual subunits are asymmetric and they polymerize in head-to-tail manner.
**Actin based motility:**

Myosins are responsible for actin based motility. Myosins belong to a family of ATP dependent motor proteins. They play a crucial role in muscle contraction, vesicle transport etc. It mainly consists of head, neck and tail domain.

There are 18 different classes of myosin. In this lecture we have discussed only a few classes of myosin.

Myosin I contains one heavy chain with a single motor domain. It acts as monomer and functions in vesicle transport.

But Myosin II contains two heavy chains. The motor domain of each heavy chain has N terminal head domain and the C-terminal tail has coiled like morphology. Motor domain catalyzes the hydrolysis of ATP & it interacts with actin.

The structure of Myosin is given below:
How do molecular motor works?

**Myosin cycle:**

![Myosin cycle diagram]

In the first step, ATP binds to myosin and induces the opening of actin binding. ATP binding causes a conformational change that causes myosin to let go off actin. ATP is then hydrolysed inducing a conformational change into high energy states (cocking), and these results in myosin weakly binding actin at a different place on the filament. Phosphate release causes myosin to bind very strongly with actin. Then ADP is released. ADP dissociation leaves the myosin head tightly bound to actin. Actin functions as nucleotide exchange factor.

**Methods:**

There are several biophysical and structural approaches which have been applied to understand the mechanism of molecular motors. Here the aim is to identify all the intermediate conformational states during one ATP cycle. One of the suitable techniques is X-ray crystallography. This allows the visualization of protein 3D structure at the atomic resolution. But it is not possible to examine the motor track interaction with the help of X-ray crystallography. To make this possible electron microscopy has been introduced.
Studies of movement due to a single myosin molecule:

In this case a special type of setup is used where “optical traps” are created by focused laser beams. These optical traps can hold small objects and by adjusting the intensity of the laser beam the force can be controlled.

Actin filament is placed in optical trap via one or two attached beads.

Myosins are kept at low concentration so that only one myosin contacts the actin filament.

ATP is also kept low so that only one ATP binds to each myosin head.

In vitro motility assays:

In vitro motility assays provide an important approach to study the dynamics of motor protein movements. It is very useful to investigate myosin function using a small number of purified components. Now-a-days a special kind of in vitro assay has been developed in which fluorescently labelled actins filament were found to move over the glass surface coated with myosin. Using this assay it is possible to show that single headed myosin will support movement. In vitro motility assays can also measure the physical and mechanical properties of single molecule to establish the fundamental properties of these proteins.

There are two typical geometries that are used for in vitro motility assays: bead assays and surface assays. In case of bead assays, filaments are attached to a substrate, such as a microscope slide, and motors are attached to a small plastic bead, generally 1 μm in diameter, or to the tip of a fine glass needle. Light microscope helps to observe the motion of the beads.
or of the needle along the filaments in the presence of ATP. The position and movement of the beads or the needle are measured photo-electrically and can be determined with a resolution on the order of nanometres and sub-millisecond time-response. In case of surface assays, the motors themselves are attached to the substrate, and filaments are found to diffuse down from solution, attach to and glide over the motor-coated surface. This can be observed using dark-field or fluorescence microscopy.

In vitro motility assays. (a) The motile activity can be detected by attaching the motor to a bead and then allowing it to interact in an ATP-dependent manner with microtubules or oriented actin filaments on the cover glass surface. (b) Alternatively, individual fluorescence labelled actin filaments can be observed moving over a lawn of myosins and microtubules can be observed moving over a lawn of dynein or kinesin motors.