Motor proteins as cargo transporters

The **cytoskeleton** superhighway: the network of protein filaments that make up the cell’s scaffolding.

*fibroblast cells labeled to show actin filaments and focal adhesions*

[Singh *et al.*, Nature Methods (2013)]
Myosin V motor protein

Essentially repurposing (and tweaking) the Myosin II biochemical cycle for a cargo transport along actin:
Myosin V motor protein

tail attaches to cargo
lever arm with six bound CaM chains
direction of motion
motor head
actin filament

36 nm

[References]
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Myosin V

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Myosin V

Direct visualization of myosin walking using high-speed AFM
Kodera et. al., Nature (2010)
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Observed hand-over-hand forward stepping (previously known) and foot stomping, where one head detaches and reattaches to same site.
Myosin reaction cycle: forward stepping

- two possible preferred angles of the head-arm junction:
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waiting state

diffusion along with ATP hydrolysis, recovery stroke

trailing head recovery stroke
Myosin reaction cycle: forward stepping

After the recovery stroke, the \(-\) orientation of the free head favors binding to forward site

Myosin reaction cycle: forward stepping

1. **ADP waiting state**: The myosin head is bound to ADP and ATP, ready for the next reaction.
2. **Diffusion along with ATP hydrolysis, recovery stroke**: The myosin head diffuses while hydrolysing ATP to ADP and inorganic phosphate (Pi). This releases energy for the recovery stroke, allowing the head to detach from the actin filament.
3. **Attachment to next actin binding site**: The myosin head then attaches to the next actin binding site, and the process repeats.

**Key Steps**:
- **ATP → ADP + Pi** (phosphate release and power stroke)
- **Leading head power stroke**: This is the force-generating step where the myosin head moves forward, propelling the actin filament.

The diagram illustrates the key stages of the myosin reaction cycle, highlighting the transitions between the waiting state, diffusion with ATP hydrolysis, and attachment to the next actin binding site.
Myosin reaction cycle: forward stepping

Two mechanisms that favor processive unidirectional motion:

1. **leading head power stroke**: biases the diffusive search for target site in the forward direction.

2. **trailing head recovery stroke**: gives trailing head correct orientation for forward binding.
Myosin reaction cycle: alternative kinetic pathways

forward step

trailing foot stomp
Myosin reaction cycle: alternative kinetic pathways

**forward step**
- 12 s\(^{-1}\)
- ADP → ADP

**trailing foot stomp**
- 12 s\(^{-1}\)
- ADP → ADP

**leading foot stomp**
- 1.5 s\(^{-1}\)
- ADP → ADP

**backward step**
- 1.5 s\(^{-1}\)
- ADP → ADP
Single-molecule experimental data
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A. Step ratio $P_b/P_f$

B. Run length $z_{run}$ [µm]

C. Velocity $v_{run}$ [nm/s] vs. Force $F$ [pN]

- Clemen et. al. (2 mM ATP, 25 mM KCl)
- Sakamoto et. al. (2 mM ATP, 150-400 mM KCl)
- Baker et. al. (1 mM ATP, 25-100 mM KCl)
- Pierobon et. al. (2 mM ATP, 25 mM KCl)
- Pierobon et. al. (in vivo)

- Kad et. al. (100 µM ATP)
- Mehta et. al. (1 mM)
- Uemura et. al. (1 mM)
- Gebhardt et. al. (1 µM)
Single-molecule experimental data

**A**

- **Step ratio $P_b/P_f$**
- **Fstall**
- **Kad et. al. (100 µM ATP)**

**B**

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**C**

- Clemen et. al. (2 mM)
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**Right panel**

- Stepping at zero load:
  - Dunn & Spudich

- Average of 231 single steps