ML-18
The Cell as a Machine

Other Motors

We have concentrated our discussion on the myosin and kinesin motors because the most is known about them. They illustrate two important regimes the highly organized skeletal muscle regime, non-processive, and the processive motor regime in which the motor remains attached to the filament. From a broader perspective, the motors in the nucleus (synthesizing RNA and DNA) and in protein synthesis are the most active during the cell cycle whereas myosin and kinesin are critical in the specialized muscle and neuron cells.

Custom Design of Motors for the Function

There are a number of important aspects of motor function that can be modified to adapt the motor to best perform the task at hand. A wide variety of cellular functions require force generation or transport, the major functions of motor proteins. For example, DNA unwinding requires a helicase, protein translocation into the ER lumen requires a chaperone protein to fold the newly synthesized protein, the proteasome unfolds proteins as part of their degradation, the Fo/F1 ATPase is a rotary motor, and a number of transport events are driven by motors that move on actin filaments or microtubules.

Efficiency of Motors: The energy of ATP in cells is about 80 pN nm whereas kT is about 4 pN nm. In terms of efficiency, from 50-100% of the ATP energy can be captured for productive work.

Tailoring to the Task

If we think about a given function, there is are obvious features of a motor that would be useful to complete the task. Often the requirements are quite different for different functions and motors will naturally adapt to optimize function. Major functions of cells involve the contraction of cytoplasm, movement in muscle, transport of vesicles, breaking filaments (disassembly), unwinding of polynucleotides, movement on polynucleotides, retraction of pili, translocation of proteins into mitochondria or ER, unfolding for protease digestion or generation of ATP.

Contraction of cytoplasm

If you take cytoplasm and allow the actin filaments to assemble with ATP, often myosin motors in the mix will produce a contraction.

Processivity versus Recruitment

Other linear motors

DNA and RNA Polymerases

Problem is to copy with high fidelity 2m of DNA that is packaged in a nucleus of 5 microns in diameter.

Physical properties of DNA (0.3 nm/base pair) double stranded helix (lots of twist, torque problems).

DNA Helicases

Unwind DNA

Condensins

AAA ATPases

Dynein

Katenin

NSF

Bacterial pilin subunits

Fo/F1 ATPase

Bacterial Flagellar Rotor

Motor Kinetics

In our discussion of the motility cycle, there were several different motor states and the kinetics of the transitions from one state to another was critical for the motility to occur. Physical chemistry of kinetics is useful to try to understand how the transitions between the states can be defined.

First order kinetics: In the case of a unimolecular complex that undergoes a reaction, there is a first-order decay process.

$$M(ATP) \text{ --- } M(ADP\text{-}Pi)$$

$$d[M(ATP)]/dt = k_1[M(ATP)]$$

Thus, if we integrate, we find that the change in concentration over a time, t, is

$$[M(ATP)]_f / [M(ATP)]_i = \exp(k_1 t)$$

Second order kinetics:

Reactions in the myosin cycle

$$M + ATP == M(ATP)$$

$$d[M(ATP)]/dt = k_2\,[M][ATP]$$

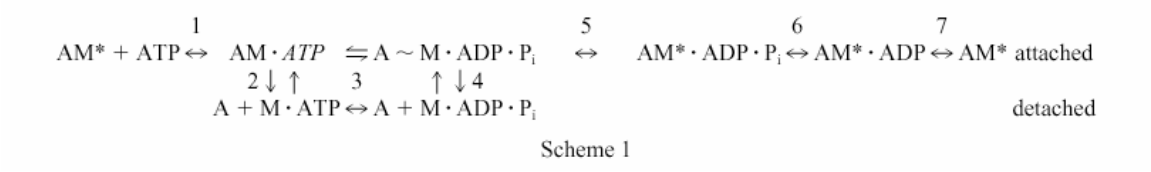
In this case, we typically define conditions such that one of the components is in vast excess ([ATP] is constant in this case) and therefore does not change in concentration. Further, we know that $[M] + [M(ATP)] = M_o$, where M_o is the original concentration of motor, M. Under those circumstances, the equation reduces to a first order differential and can be solved for a given concentration of ATP.

$$d(M_o - [M])/dt = k_2\,[M][ATP]$$

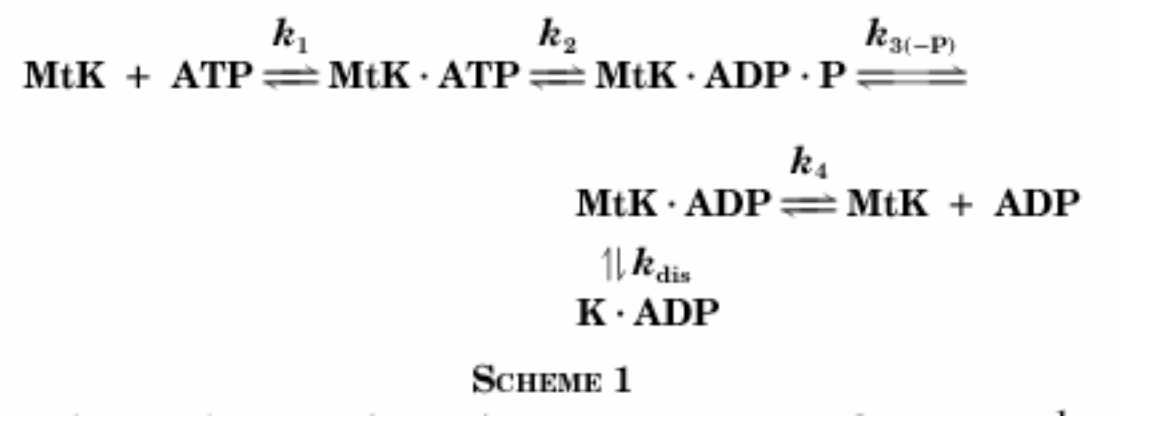
or $$-d[M]/[M] = k_2\,[ATP]\,dt$$

upon integration $$[M]_t/\,M_o = \exp -(k_2\,[ATP]t)$$

Now we can look at the myosin and kinesin reaction cycles and see how measurements of



Scheme 1



SCHEME 1

Problems:

1. We discussed processive motors in terms of the kinetic scheme for kinesin (Scheme 1 with MtK above). If we assume that the concentration of ATP is high, then ATP binding will not be the rate-limiting step (K_1). What is the rate-limiting step, if $K_2 = 1000\ s^{-1}$, $K_3 = 100\ s^{-1}$, and $K_4 = 400\ s^{-1}$? If the step length is 8 nm/ATP, then how fast can the motor move if it has one head (assume that between step 3 and 4, the kinesin head will come off the microtubule)? (same question for 2 heads like the normal molecule?)
2. (Extra Credit 10 Points) For muscle myosin, which is non-processive (see scheme 1 with AM above), the rate-limiting step is the hydrolysis of M.ATP to M.ADP.Pi (this is the forward rate constant of #3, which is typically $40\ s^{-1}$). M.ADP.Pi then binds to actin to produce a force by the swing of the crossbridge, Pi and then ADP are released. Myosin bound to actin then waits for another ATP to release it and start the cycle again. The series of steps from force production to release takes about 2 milliseconds without load in a maximally activated muscle. Assuming 0.002 sec is the time that the active heads are bound to the actin filament and that the forward swing of the crossbridge is 10 nm, then what is the maximal velocity of muscle contraction (you should also assume that without an external load, the myosins are pulling against themselves; i.e. half of the time is spent producing force to pull other myosins forward and half of the time is spent being pulled forward by other myosins).
3. (Extra Credit 10 Points) What happens to the time of force production in the muscle described in Problem 2, when a load is applied that slows contraction? Assuming that the velocity is slowed to 1/10 of the maximal velocity determined in Problem 2, what is the average time that a head is bound and producing force before it is released? For an additional 5 points, compute the fraction of the myosin heads (on average) that are bound to actin under these conditions.