Chapter 7 Odd Solutions

1. The answer expected for this question is that random diffusion is a good way to travel short distances, but a poor way to travel long distances, because the time taken goes up as the square of the distance. Therefore for short distances there is no need for a motor at all.

A variety of secondary answers are possible. For protein-sized molecules, and even for objects several orders of magnitude larger, objects in water have essentially zero inertia. Viscous drag means that a molecule will stay roughly where it is moved to; it will not 'continue to move unless acted upon by another force' (as in Newton's first law of motion). On the other hand, thermal motions ('Brownian motion') for molecules the size of proteins can be very significant: a protein will jiggle around and deform extensively merely because of bombardment by solvent. Therefore you cannot hope to move a protein by 'pushing' it: you need to allow it to move around by thermal motion and then prevent it from moving back again (a ratchet mechanism, which as described in the chapter requires an energy input). Thus, over short distances the driver is thermal motion, energy being required to keep the molecule where it got to; over larger distances the driver is a more direct mechanical movement.

3. To answer the questions in the order asked:

A nucleoside is an obvious choice at an early stage in life where nucleotides were the dominant complicated molecules present; as noted elsewhere, a large proportion of signalling molecules and cofactors are based on nucleotides. The spontaneous rate of phosphate hydrolysis is extremely slow, so kinetically nucleoside phosphate hydrolysis is a good choice, as the signal will not get degraded unless the cell wants it to be. On the other hand, enzymes can catalyze phosphate hydrolysis at a usefully fast rate. And of course a phosphate is a rather easy object to recognize, mainly because it has a charge, so that development of a switch based on the loss of a phosphate is perfectly feasible. Guanine is a purine and is therefore larger and easier to recognize than a pyrimidine. The other purine is adenosine, which could also have been used (and is indeed used, as discussed in Chapter 7). One could argue that adenosine is likely to be less desirable as a signal, because its 'main' use is in energy storage. There is nothing particularly good about the third phosphate of GTP: hydrolysis of GDP to GMP has almost the same standard free energy change. However, GTP is at 10 times the concentration of GDP, and GMP is at even lower concentrations; it therefore makes more sense to use the more common molecule, because a signalling mechanism based on the conversion of GDP to GMP would require additional rapid recycling of the GMP. The hydrolysis of GTP to GMP would liberate more energy (particularly when the pyrophosphate liberated is rapidly removed, as normally happens in the cell). However, it is wasteful to do this if the extra energy is not needed; one can argue that because only a single phosphate is removed, this shows that the switch is as 'ideal' as it needs to be.

5. In resting muscle, tropomyosin overlays the myosin-binding sites on actin, and is locked down in this position by troponin T and troponin I. Upon release of calcium from the sarcoplasmic reticulum, calcium binds to troponin C, which unlocks tropomyosin from actin, allowing myosin heads to access the binding sites on actin. Once one myosin head has bound, this fully displaces tropomyosin and allows additional myosin heads to bind, initiating muscle shortening and contraction. Once calcium has been pumped out of the cytoplasm and calcium levels have returned to normal, tropomyosin again binds to actin, preventing myosin from binding.

The role of titin is quite different: it forms essentially a long piece of elastic, which attaches the myosin bundle to the Z disk (see Figure 7.11).

7. The basic structure of intermediate filaments (IFs) is a globular head and tail, joined by a long helical section, which forms a coiled-coil dimer, with the heads in register. These dimers then assemble into tetramers, composed of an antiparallel staggered pair of dimers, making a symmetrical unit. This is of course different from actin and tubulin fibers, which have an important polarity; there can therefore be no directed movement along IFs. These tetramers can then assemble into much longer fibers. The best-known IFs are the keratins, found in hair, nails, and horns. However, most IFs are intracellular assemblies that maintain cellular structure. These include vimentin, a common IF in many cell types used to maintain cell shape and locate internal organelles; desmin, a component of the sarcomeres in muscle cells; neurofilaments, which provide structure to nerve axons; and lamins, used to provide structure particularly within the nucleus. Unlike actin and tubulin, IFs can stretch, to several times their original length if required. Mutations in IF genes lead to a variety of diseases.

Unlike actin and tubulin fibers, IFs are not highly dynamic, nor are their assembly and disassembly regulated by nucleotide phosphates. They are thus mainly a static structural component. They do, however, get remodeled fairly rapidly, apparently mediated to a large extent by dynein and kinesin, which can transport IF precursors along microtubules [see, for example, B.T. Helfand, L. Chang and R.D. Goldman, *J. Cell Sci.* 117:133–141, 2004].

The last part of the question contrasts the assembly and components of actin and tubulin fibers with IFs, and the discussion of **Fibrous proteins (*1.14)** and Section 1.3.2 are relevant. The point made there is that actin and tubulin fibers need to assemble and disassemble continuously, and are therefore made from globular monomers that are easy to put together into filaments and take apart. By contrast, the really fibrous structures such as silk and collagen are permanent fibers and are made from molecules that are themselves long and fibrous. IFs are much more like collagen in this regard, because they are mainly long-term structural components, which is consistent with their being made from fibrous monomers.

9. Post-translational import is most common across the yeast ER membrane and in bacteria. In post-translational import, the protein synthesized is ultimately going to end up as a globular folded protein. It is therefore not stable as an unfolded protein and needs to be covered by chaperones, typically the Hsp70 chaperone, to prevent it from aggregating. Thus, as the protein is expressed, it gets coated by chaperones as it emerges from the ribosome. The N terminus of the protein is subsequently recognized and the protein is threaded into

a specialized complex containing Sec61 together with several other proteins. As the protein emerges into the ER it is coated by another chaperone called BiP, which is related to Hsp70; the additional proteins complexed to Sec61 aid in attaching BiP to the emerging chain. Directional movement into the ER is accomplished by dissociation of the chaperone from cytoplasmic protein, a process requiring the hydrolysis of bound ATP from Hsp70. This uncovers a length of protein, which is then free to move in and out of the Sec61 channel. When a long enough length has moved into the ER, it becomes coated by BiP inside the ER, which then prevents it from moving back out again—another ratchet mechanism [K.E.S. Matlack et al., *Cell* 97:553–564, 1999]. Finally, BiP dissociates from the protein as a result of hydrolysis of bound ATP to ADP. It is therefore this hydrolysis that ultimately provides the energy for translocation.

One Hsp70 molecule binds to about seven amino acid residues (see, for example, the Wikipedia article on Hsp70). Therefore a peptide fully coated by Hsp70 or BiP has approximately one chaperone per eight residues. Translocation of a peptide into the ER requires the binding and dissociation of Hsp70 at one side of the ER membrane and of BiP at the other, and therefore requires two molecules of ATP to be hydrolyzed per eight amino acids. Post-translational import therefore requires an extra 0.25 ATPs per residue compared with co-translational import. Co-translational import requires (a) attachment of the amino acid to a tRNA, which involves the hydrolysis of a trinucleotide to a mononucleotide and therefore uses up essentially two phosphates; (b) attachment of the amino acid to the growing peptide chain, accomplished by EF-Tu with the accompaniment of hydrolysis of GTP to GDP; and (c) translocation of the ribosome to the next codon, accomplished by EF-G with the accompaniment of hydrolysis of GTP to GDP. Translation therefore takes approximately four phosphates per amino acid (not counting initiation and termination). Thus, co-translational import requires roughly four phosphates per residue, whereas post-translational import requires 4.25, an insignificant difference. [For further details on both mechanisms see, for example, A.K. Corsi and R. Schekman, *J. Biol. Chem.* 271:30299–30302, 1996.]

N1. The distance across an oocyte is larger by a factor of 100, so the time is longer by a factor of 10^4; that is, 1 s. Along an axon is a factor of 10^6 in distance, or 10^{12} in time, corresponding to 10^8 s or about 3 years (as mentioned later in the text).

N3. A complete rotation of the ring generates 3 ATP molecules and requires the passage of n protons. Therefore the number of ATPs produced per proton is $3/n$. With $n = 10$, you get 0.3 ATP per proton, but with $n = 14$, you get only 0.21 ATP per proton. If the aim is simply to produce as much ATP as possible per proton, then it is surely better to have $n = 10$. The question is therefore why some organisms should use larger values of n.

The answer is outlined at the end of Section 7.2.6. There needs to be some excess of free energy to make sure that the reaction goes completely and is not allowed to occur backward. This means that if you want to make sure that protons produce ATP and not the other way round, you need to make sure that there are relatively large numbers of protons that make 1 ATP. This is equivalent to going uphill in a low gear (many rotations of the engine for the same distance traveled). Therefore presumably the number of protons per ATP is in some way evolved to be optimal for the growth conditions of the organism.