Chapter 8 Odd Solutions

1. The basic answer is no. Most organelles seem to operate more or less autonomously. A cell has an important need to be able to respond to its environment, otherwise sooner or later it will die: hence the need for efficient signaling across the cell membrane. The inside of a cell is, however, kept rather constant, and thus the major need for signals, namely to regulate what goes on inside in response to changes outside, is not present. There is of course a need to regulate the rate of what the organelle does, but this can be done largely by regulating the supply of metabolites. For example, the mitochondrion operates mainly as an energy generation system: metabolites go in and energy comes out. All it therefore needs is a supply of metabolites, and it produces a supply of energy in proportion. There are membrane transporters for equilibrating metabolites across the membrane, and there are also (as we see in Chapter 7) systems for getting proteins into the mitochondrion. However, there seems to be very little need for signals to pass across the membrane. It should nevertheless be noted that, in contradiction to the statement just made, there **is** a system for signaling mitochondrial dysfunction to the nucleus. In yeast it is controlled by the Rtg2/Mks1 (retrograde regulation) complex.

3. (a) Calmodulin has two highly homologous halves. Each half contains two EF-hands (as described in Chapter 1), which consist of two helices linked by a loop. Calcium binds to residues in the loop and at the ends of the helices. The linker between the two halves is a continuation of one of the helices. In the crystal structure of free calmodulin, this is a continuous helix, but in solution it is flexible, not being really helical at all. When bound to a ligand, the central helix is completely destroyed and the protein folds in half, wrapping itself around its ligand, so that both halves contribute to the binding interface. This creates a strong binding interaction. (b) When calcium binds to the EF hands, there is a reorientation of the adjoining helices, which alters calmodulin from being a fairly hydrophilic convex shape to one with two marked hydrophobic cavities. (c) These cavities are lined with methionine residues. Methionine has a long hydrophobic side chain, which makes it possible to adjust to a wide range of different hydrophobic ligands. Moreover, the sulfur is very polarizable, meaning that it will interact favorably with polar ligands too. It is no coincidence that several proteins that need to be able to bind to a wide range of hydrophobic ligands have methionine-rich binding sites. As well as calmodulin, these include the signal recognition particle and nuclear import/export proteins. (d) Calmodulin often binds to peptides in a helical conformation, with methionine residues binding to hydrophobic side chains and positively charged residues on the peptide interacting with acidic residues on calmodulin. There are two large hydrophobic cavities in calcium-bound calmodulin, which often bind large hydrophobic side chains such as Trp or Leu. skMLCK (myosin light-chain kinase) binds in the more normal orientation, with the N-terminal end of the α helix binding

to the C-terminal domain. However, melittin binds in the opposite orientation, with its C terminus binding to the C-terminal domain.

5. An antibody with a single antigen recognition site would decrease the efficiency of signaling involving that receptor, because receptors bound to antibodies would not be able to bind ligand and dimerize. However, the more normal antibody containing two antigen recognition sites would cause dimerization in the absence of ligand and thus create a signal. Such events have been confirmed experimentally.

7. Because lipid rafts are difficult to characterize or measure, the most convincing evidence comes from studies in which lipid rafts have been experimentally removed or decreased in concentration. Probably the best way to do this is by decreasing the concentration of cholesterol in the membrane, because a high concentration of cholesterol (up to 50% by number of molecules) seems to be crucial for maintaining the integrity of lipid rafts. This can be done for example by adding β-cyclodextrin, which complexes to the cholesterol and probably does not interfere in other ways. For example, depletion of cholesterol from mast cells leads to reduced IgE signaling. There is abundant evidence that some signaling pathways are localized to particular regions of the membrane, but little solid evidence that these can be equated with lipid rafts.

9. In most cases, it leads to the overproduction of red blood cells such that those affected can carry a higher concentration of oxygen in the blood. The cross-country skier Eero Mäntyranta won seven medals at winter Olympics, partly as a result of this mutation. Remarkably, the mutation seems to be almost entirely benign [A. de la Chapelle, A.-L. Träskelin and E. Juvonen (1993) *Proc. Natl. Acad. Sci. USA* 90:4495–4499].

N1. If the activated switch is only on for 10% of the time, the inactive switch is on for a factor of 1000 times less, or 0.01%. We need some way of converting from the 10^{-4} per plant per year for a nuclear accident to the equivalent frequency for a cell. One obvious way is to do this in ratio to the lifetime of the nuclear plant and the cell. If we assume that a nuclear plant has a lifetime of about 30 years, and a eukaryotic cell has a lifetime of 30 weeks (very variable, depending on what type of cell), then our 'severe core damage' to the cell should be less than 10^{-4} per cell per week. If an inactive switch is in fact open for 0.01% of the time, this implies that it is capable of transmitting an incorrect signal at this frequency, which is markedly higher than our figure of 10^{-4} per cell per week. Given that the overall risk is the frequency of occurrence multiplied by the risk to health of an accident, then one must conclude that the 'risk to health' of an accident is vastly less than it would be for a nuclear leak. In other words, and assuming that evolution has arrived at something close to the best solution (a reasonable assumption), it means that the 'risk to health' to a host organism of an incorrect signal being transmitted is vastly less than the risk to health of a nuclear leak. The 'risk to health' of the individual cell's receiving the incorrect signal is in fact very large: it could very easily start doing something entirely inappropriate as a result of the signal's being switched on, because in some cases the activation of even a single receptor is enough to activate an intracellular response. It must therefore be true that the host organism has to have a very effective means of 'isolating' cells that misbehave. That is, the body must have an almost foolproof

mechanism for detecting and destroying inappropriately activated cells. This is basically what the body does continually in detecting and destroying potentially cancerous cells before they have a chance to divide, and the calculation above shows what an effective mechanism this is most of the time.