Chapter 4

Epilogue

The lesson we have received time and again during the course of the work leading to this thesis is that it is futile to turn to "model" membranes to understand the nature of the surface of a living cell. The levels of organisation we have observed on the surface of a mammalian cell cannot be assembled piecemeal on a synthetic bilayer, the levels are created and maintained on the surface by cues from within the cell, and extracellular agents enlist the help of such an organisation to transmit matter and information into the cell. Ongoing work in our group and elsewhere will reveal the nature of the cues and show how the patterns on the surface respond to the world outside to send a signal within.

4.1 Short range order on the plasma membrane

The hundred of kinds of lipids that form the eukaryotic plasma membrane has plagued biologists with the question: is this variety only a result of historical contingency? The diversity is not in the shape, size and charge of the hydrophilic head group alone but also in the length of the fatty acid chains, their shape and rigidity (governed by the number and position of unsaturated bonds), and even in their number (lipids with very short head groups and a triplet of hydrophobic chains have been found in special locations of the membrane). It is known that, in order to function, some proteins in the membrane need to be surrounded by a shell of phospholipids with a specific head group; and also that the cell adjusts the composition of its lipids with different conformations of fatty acid chains so that the membrane is always in a fluid state whose viscosity is within prescribed limits (Alberts et al; 1994). Yet the question remains — does the cell really need all the lipids comprising its membrane?

It is pleasing to note that we have put a small piece of the puzzle in place by demonstrating that a novel kind of short range order on the surface of the cell is maintained by the interaction of a specific pair of components of the membrane —- cholesterol and sphingolipid. With GPI -anchored proteins of wildly varying ectodomains but a constant sphingolipid tether we have shown that they exist in extremely dense and small clusters (less than 4 molecules in a diameter no more than 50 Å) and that the level of clustering

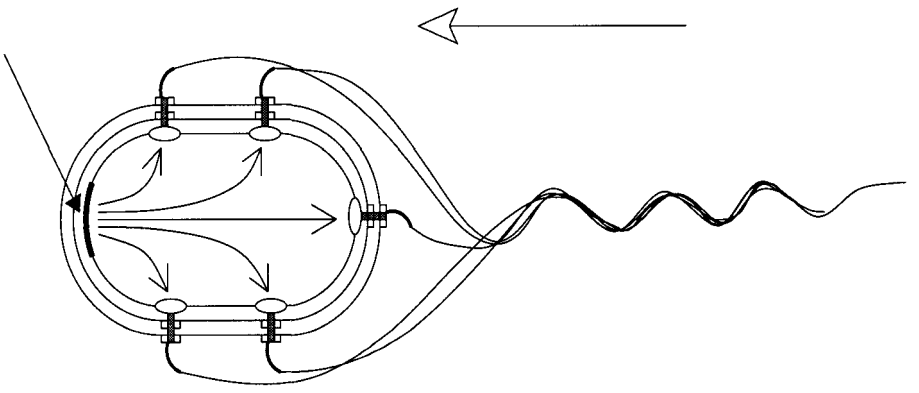

Figure 4.1: Receptor clusters in the plasma membrane of a prokaryote. A chemoreceptor is clustered at one pole of E coli, the black arrow aims at the "nose" of the bacterium. Arrows within the cell represent signals transmitted from the "nose" to the flagellar motors when the chemoreceptors bind to the ligand. The signal makes the motors rotate anticlockwise so that the bacterium moves in the direction of the open arrow (Parkinson and Blair; 1993).

is tuned by the amount of cholesterol in the membrane. Our observation is consistent with that of Friedrichson and Kurzchalia who have detected oligomers of GPI-anchored proteins by presenting the surface of a cell with a chemical crosslinker of spacing 11 Å (Friedrichson and Kurzchalia; 1998). These results are also consistent with the studies of single-molecule epifluorescence conducted on a GPI-anchored protein that reported fast brownian motion of most molecules but a small fraction (between 6 % and 20 %) diffusing at a much slower rate (Vrljic et al; 2002). Though these studies were unable to characterise the size or the origin of the slowly diffusing species we surmise that the sluggishness is owing to the oligomerisation of the proteins. Our experiments corroborate the result that only a part of the population of GPI-anchored proteins on the surface (about 20 %) exist in clusters, the rest being in the form of monomers.

We do not yet know the precise role of cholesterol in the formation of the clusters — is one or more molecules of cholesterol physically a part of the tight cluster or does cholesterol create a local environment in the plane of the membrane that favours oligomerisation of the protein without itself being incorporated in a cluster? What are the forces binding the molecules in an oligomer? Even more pressing is the question: what are the clusters there for?

The remarkably sophisticated chemotactic behaviour of a bacterium, Escherichia coli, offers insight into the potential role of protein clustering in the life of a cell. An eukaryotic cell can sense chemical gradients by comparing concentrations at different parts of its body, but a bacterium, much smaller and more agile, has to get its bearing in chemical gradients by measuring changes in concentration with time as it moves about. An E coli swims about 10 to 20 body lengths per second, by comparing current chemoreceptor occupancy with that during the previous few seconds, the bacterium is able to take measurements over distances of many body lengths (Parkinson and Blair; 1993). E coli can

respond to extremely low concentrations of chemical attractants, concentrations of less than 5 nM in the case of aspartate (corresponding to a separation of 1 μm between the ligand molecules in the solution)! It can also sense chemical gradients extending over five orders of magnitude in the concentration (up to 1 mM). Maddock and Shapiro visualised the aspartate receptors of E *coli* with gold-labeled antibodies and thin-section electron microscopy (Maddock and Shapiro; 1993). They found that over 70% of the receptors were in clusters, frequently toward one pole of the cell (Figure 4.1).

Based on theoretical modelling, Bray, Levin and Morton-Firth have proposed an explanation of the need to have receptor clusters in E *coli* (Bray et al; 1998). The theory is so simple that the model can easily be generalised to understand the effect of clustering in other cells and in contexts other than chemotaxis. The principal assumption of the model is that a receptor occupied by a ligand is able to infect a neighbouring receptor in a cluster and thus increase the probability of the neighbour triggering a downstream signal even if the neighbour is not bound to the ligand. With this model of cooperative activity, it is easy to show that increasing the extent of spread of activity through a cluster of receptors increases the sensitivity to extracellular ligands but severely diminishes the range of concentrations of the ligand over which the cell can detect gradients. However, a combination of low threshold of response and wide dynamic range can be attained if the cell has both clusters and single receptors on its surface, particularly if the level of cooperation can adapt to external conditions.

Indeed we have evidence to suggest that the clusters can rearrange themselves to adapt to external stimuli. Though multiple species of GPI-anchored proteins inhabit the same cluster, presenting the cell surface with an antibody specific to a single species leads to the segregation of that species into cross-linked patches while the rest of the species reorganise the a *priori* cluster at the nanometre scale. The forces binding the proteins are likely to be weak, over a short range cross-linked proteins can be induced to detach and reorganise into distinct structures. The adaptability of the organisation will be enhanced if several clusters find themselves close to one another, like the occupants of an island in a sea of monomers.

4.2 Higher level organisation

Any mechanism for the formation of the clusters must be consistent with the following features: (A) the capacity of the clusters to exchange their constituents, and (B) a constant proportion, over a large range of expression, of the proteins forming clusters, the rest being monomers. (A) and (B) are in apparent contradiction because dynamic exchange will result in chemical and thermal equilibrium while a constant proportion of monomers and clusters indicate that this system is far from equilibrium. This contradiction may be resolved if we stipulate the presence of actively generated domains on the membrane that pose a barrier to clusters drifting away from the domains, and hence prevent the

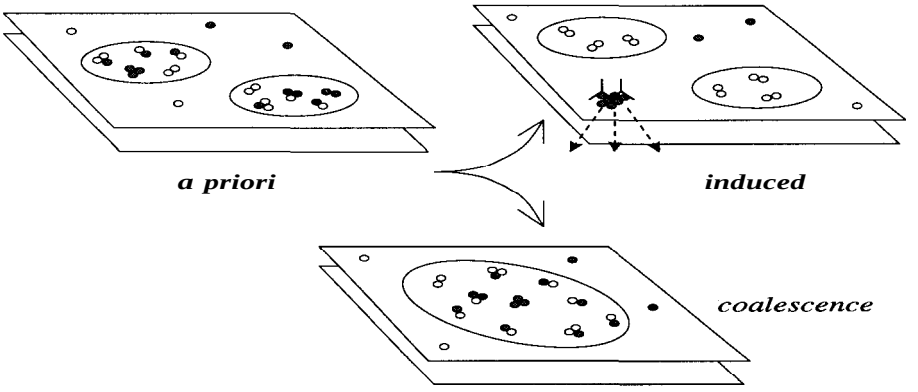

Figure 4.2: A priori and induced structures on the cell surface. White and grey dots represent two species of GPI-anchored proteins, each pair of rectangles represent the exoplasmic (top) and the cytosolic (bottom, partly hidden) leaf of the plasma membrane. Circles on the surface are rafts where GPI-anchored proteins are clustered at the nano scale; outside the circles the proteins exist as monomers. Both grey and white dots inhabit the nanocluster. Upon induction of a larger scale clustering of the grey dots by an antibody, rafts expel the crosslinked species. Broken arrows represent the potential of the crosslinked species to transduce a signal into the cell. Rafts can also coalesce to form bigger and more stable structures such as caveolae.

attainment of equilibrium. This would predict that the ratio of clusters to monomers is set by active processes and cholesterol homeostasis regulates this activity. Although the level of sphingolipids does not directly affect the relative population of proteins in clusters, the enhanced susceptibility of these clusters to disruption following the removal of cholesterol in cells depleted of sphingolipids suggests that both sphingolipids and cholesterol are involved in this higher level organisation. In sphingolipid-depleted cells other lipids with saturated fatty acid chains may substitute, though poorly, for the role of sphingolipids.

We do not yet know the size of the domains (or rafts) that prevent mixing. These structures must be as large as to accomodate a single cluster (~ 50 Å) but not too large, otherwise they would have been detected by fluorescence microscopy. If the rafts contain many clusters they must do so at low enough density to prevent resonance amongst fluorophores belonging to separate clusters. The structure of the rafts, their stability, and their response to extracellular stimuli await discovery through further experiments.

4.3 Consequence of clustering and super-organisation

In chapter 1 we have seen that GPI-anchoring is a necessary motif in targeting proteins to a clathrin-independent endocytic route. Upon crosslinking, GPI-anchored proteins cease to follow this route, instead they are internalised through clathrin-coated pits. We believe that clustering is the signal to trigger the endocytic machinery but in order to be noticed the signal has to cross a threshold. Super-organisation into rafts enables the clusters to generate an appreciably strong signal for endocytosis. Akin to the strategy of chemotactic response in E *coli*, the pooling of small and dense clusters from a sea

of isolated proteins into malleable and mobile organisations (the rafts), enables the cell to combine the opposing demands of extreme sensitivity and a wide dynamic range of response into an optimum. Not only ligands (such as folate), but many parasites (viral, bacterial and protozoan) use GPI-anchored proteins as their receptors and possibly exploit the hierarchical organisation of this protein to enter the host.

Many proteins that glue neighbouring cells to mould a tissue are GPI-anchored. The presence of multiple GPI-anchored proteins in a single cluster has the potential of tuning the specificity of adhesion of neighbouring cells (Harris and Siu; 2002).

Even more significantly, this nanoscale clustering is likely to be utilised in the conversion of prion proteins to infectious scrapie. After the cellular isoform of the prion protein (PrP^C) is synthesised in the endoplasmic reticulum, it transits through the golgi apparatus to the plasma membrane to which it is bound by a GPI-anchor. Efficient formation of the scrapie isoform of the prion protein (PrP^{Sc}) requires the targeting of PrP^C by its GPI-anchor to caveolae-like domains. Redirecting $\mathbf{PrP^C}$ to clathrin-coated pits by a modification of the lipid anchor prevents the formation of PrP^{Sc} (Kaneko et al; 1997). Cholesterol depletion too inhibits the transformation of $\mathbf{PrP^C}$ into $\mathbf{PrP^{Sc}}$ (Taraboulos et al; 1995). We propose that the nanoscale clusters of prion proteins could act as high-affinity receptors for vanishingly small levels of infectious scrapie particles. The rafts could provide a rich source of prion in the plane of the membrane required for efficient conversion to scrapie, the isolated monomers being a constant substrate.

4.4 Revision of the fluid mosaic model

The view of the plasma membrane as a passive matrix of lipids supporting proteins that control the state of the cell is no longer tenable. Lipids actively organise proteins on the surface of the cell. The intracellular organelles have distinct compositions of the enzymes in their lumens and also of the proteins and lipids forming their membranes. Not surprisingly, lipids, along with proteins, are sorted at the surface in the process of endocytosis.

We have demonstrated a hierarchy of organisation on the plasma membrane (Figure 4.2). The a priori structures consist of nanoscale oligomers of proteins held by cholesterol. Then the cell organises these clusters into larger structures whose character depends on the nature of the perturbation to which it is exposed and of course, on the state of the cell. Though the individual protein clusters are small (not containing more than four molecules), they can be induced by specific extracellular perturbations to form larger aggregates that are potential agents of transducing intracellular signals. The smallness of the a priori clusters could be a strategy adopted by the cell to maintain the clusters below the threshold of signalling in the unperturbed state, while the higher level organisation (raft) would enable the perturbing agent (an antibody, for instance) to cross the threshold. Depending on the function the cell has to perform, rafts may coalesce to form structures

at an even higher level of the hierarchy — these structures could be stabilised to form permanent invaginations such as the caveolae, or they could serve as platforms to trigger endocytosis.

A new model of the plasma membrane has begun to emerge in which lipids are as active as proteins in responding to the state of the cell. Such a model has to describe the lipid-dependent hierarchical organisation on the surface and the reaction of such an organisation to specific perturbations.

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