

## **Answers to home questions – Protein lateral movement in the membrane – computer tutorial**

Introduction to Cell Biology – course 72373 – 2007

- 1) Do the experiments performed with human-mouse cell fusion prove conclusively that proteins move laterally in the membrane? What other possibilities exist which can explain the experimental results (development of mosaic cells)? What experiments do you suggest to prove or eliminate the other options?**

The experiments presented at the tutorial suggest, but do not prove, that proteins move laterally in the membrane. Several possibilities exist which can explain the development of the mosaic pattern in the fused cells. For example:

- 1) Lateral movement in the membrane (our hypothesis)
- 2) Movement of proteins, from place to place on the plasma membrane via the cell interior – proteins are internalized in one place and returned to the membrane in another.
- 3) Rapid synthesis of new proteins which are then transported to the membrane
- 4) Replenishment of membrane proteins from an intracellular stock (i.e. protein in the cell which have already been synthesized but not yet transported to the membrane)

Option 3 can be tested by fusing cells in which the protein synthesis machinery has been stopped, using various pharmacological tools (Frye and Edidin did this with Puromycine, Cycloheximide and Chloramphenicol). Option 4 can be tested by inhibiting exocytosis, through which membrane proteins synthesized in the ER arrive to the plasma membrane. Frye and Edidin tried to do this by depleting the cell of ATP, which they suggest is important for insertion of proteins into the plasma membrane. Today we have better and stronger tools, such as specifically silencing genes involved in transport using techniques like RNAi. Finally, option 2 can also be tested by using either pharmacological tools (drugs) which inhibit endo- or exocytosis or silencing of the genes involved.

Please note that these treatments (silencing of genes involved in endocytosis or exocytosis, for example) can be quite harmful to the cells, and thus appropriate controls must be used. For example, inhibition of such cellular pathways may also inhibit the fusibility of the cells, or affect the allocation of membrane proteins to specific membrane domains, things which need to be tested. Since usually gene silencing is a long term treatment, it may be better to use pharmacological inhibitors (such as Brefeldin A, which inhibits transport from the ER to the Golgi) which usually act fast and can be washed away to restore normal cellular function. Alternatively, temperature-sensitive mutants in which a change in temperature blocks exocytosis, and a return to permissive temperature restores this function. Such mutants are well known in yeast (sec mutants).

Finally, there are many other, modern approaches to test this hypothesis, for example using liposomes (in which the cellular mechanisms which are relevant to the possibilities

mentioned above have been removed), well planned FRAP experiments (possibly on liposomes or membrane bilayers), etc.

**2) In table 2 of the paper by Frye and Edidin**

**([http://teachline.ls.huji.ac.il/72373/local/tutorial/A\\_1.html#time-graph](http://teachline.ls.huji.ac.il/72373/local/tutorial/A_1.html#time-graph)), we can see an odd phenomenon: While there are cells (mainly after 10-25 minutes) in which human proteins have moved but mouse proteins have not (M1/2-H1), no cells with the opposite phenotype (M1-H1/2) can be seen. What does this suggest about the lateral movement of human and mouse proteins checked? How can this phenomenon be explained?**

The fact that M1/2 – H1 cells are seen, but M1-H1/2 cells are (almost) not, suggests that the human proteins recognized by the anti-human antibodies are more mobile than the mouse proteins recognized by the anti-mouse antibodies used. From the “materials and methods” section of the original paper we learn that the anti-human antibodies were raised against whole human cells – meaning they recognize most or all of the proteins. In contrast, the anti-mouse antibodies recognize a specific type of proteins (h2k histocompatibility antigens). Frye and Edidin suggest that the difference is caused by a “concentration effect” (the anti-human antibody recognizes more antigens, so that the signal is stronger and if there is lateral movement it will be seen more easily).

Another option is that the mouse protein which the antibodies recognize is partly anchored to the membrane. In fact, the anti-mouse antibodies used by Frye and Edidin recognize a part of the MHC (Major Histocompatibility Complex) – and membrane microdomains (Cholesterol/sphingolipid rich membrane “rafts”) have recently been shown to be critical for the biological role of MHC molecules. In fact, the mouse proteins recognized by Frye and Edidin may not move randomly in the membrane after all...