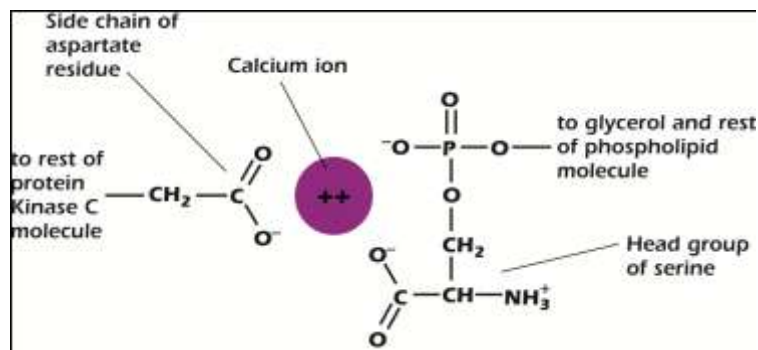


Chapter 7

Web Text Box 2

A GFP chimera used to study protein translocation

Protein kinase C (book page 265) is a serine-threonine protein kinase that translocates to the plasma membrane when the calcium concentration in the cytosol increases. Modeling had suggested that calcium ions formed a bridge between negatively charged aspartate residues in protein kinase C and negative groups on the phosphatidylserine that forms part of the plasma membrane, as shown below. Of course when calcium disappears the negative aspartate and negative lipid repel, driving protein kinase C off the membrane and back into the cytosol.



A GFP chimera used to study protein translocation

The models did not indicate which of a number of possible aspartates were the most important. Senena Corbalan-Garcia and coworkers generated a number of mutants in which individual aspartates were mutated to asparagine. Each mutant, as well as the wild type protein, were then generated as green fluorescent protein chimaeras (book page 115). Plasmids encoding the engineered proteins were transfected into rat basophil leukemia cells, a model for immune system cells. The cells were loaded with a calcium indicator dye called Fura Red, which fluoresces only when calcium concentrations are low.

We show two movies of the same four cells that were expressing wild type protein kinase C as a green fluorescent protein chimaera. Although we show the two movies independently, they were acquired simultaneously. They are speeded up considerably – each represents ten minutes real time. The first movie shows the signal from Fura Red while the second shows the signal from the green fluorescent protein.

[See Movie 7.1](#)

Upon stimulation with the agonist, the cells respond with increases of cytosolic calcium. The cells appear to flash – each time a cell goes dark in the red channel, cytosolic calcium is high. The calcium signal is generated by agonist-activated Gq which in turn activates phospholipase C β , generating IP₃ that causes calcium release from the endoplasmic reticulum (book page 254).

[See Movie 7.2](#)

When we look at the signal from the protein kinase C-green fluorescent protein chimaera, we see that the chimaera, which is initially distributed uniformly through the cytoplasm (but not the nuclei), translocates to the plasma membrane when the cells are stimulated with agonist. Comparing the two movies, we see that every time cytosolic calcium increased, the chimaera moved to the membrane, moving off again each time calcium concentrations fell.

Senena Corbalan-Garcia and coworkers found that a chimaera in which one aspartate residue (Asp187) was mutated to asparagine failed completely to translocate in response to a calcium signal. This result showed that although the other aspartates may play an important role in holding the calcium ion in place, the single aspartate at position 187 was absolutely critical for forming the bridge that held protein kinase C at the plasma membrane.

For more detail see Bolsover et al. 2003. *Journal of Biological Chemistry*, 278:10282.