3.12: Molecular Aspects of Calcium Ion-regulated Intracellular Processes (Part 2)

Sarcoplasmic Calcium-binding Protein from *Nereis diversicolor*

The calmodulin superfamily of proteins also includes' *sarcoplasmic Ca^{2+}-binding proteins* (SCPs) that can be found in both vertebrate and invertebrate muscle.[129] The function of SCPs is not yet known, but their sequence homology with Ca^{2+}-binding proteins of known tertiary structure suggests that they originally contained four helix-loop-helix Ca^{2+}-binding domains. Ca^{2+} binding has been preserved in the first and third domains of all known SCPs, but only one, if any, of domains II and IV is functional. The three-dimensional crystal structure of an SCP from the sandworm *Nereis diversicolor* analyzed at 3.0 Å resolution[130] can be seen in Figure 3.26. (See color plate section, page C-11.) The C-terminal half (domains III and IV) of the molecule contains two Ca^{2+}- binding EF-hands (green and red in Figure 3.26) similar to calbindin D_{9k} and the globular domains of troponin C and calmodulin. The N-terminal half is, on the contrary, markedly different from the normal helix-loop-helix geometry. Domain I binds Ca^{2+} with a novel helix-loop-helix conformation, whereas domain H lacks calcium-binding capacity. The two halves are packed closely together, and are not, as in troponin C or calmodulin, connected by a solvent-exposed α-helix.

Membrane Cytoskeleton and Phospholipid Binding Proteins

It has long been suspected that Ca^{2+} ions are somehow involved in exocytosis. Recently several groups[131] have isolated intracellular proteins that associate with membranes, and/or membrane cytoskeleton proteins, in a Ca^{2+}-dependent manner, and that seem able to mediate vesicle fusion or aggregation at Ca^{2+} concentrations above 200 μM. These proteins—endonexin, calelectrin, p36, and pII—have stretches of consensus amino-acid sequences that are also found in a phospholipase A_2 inhibitor protein, lipocortin.[132] It appears that further studies of this new class of proteins, known as annexins, will lead to new insights into cell-signaling pathways. Multiple functions have been proposed for the annexins, but no cellular role has yet been defined.[133] The first crystal structure of an annexin, human annexin V—which *in vitro* will form voltage-gated Ca^{2+} channels—has been determined recently.[172] In annexin, the three Ca^{2+}-binding sites are located on the side of the molecule that is involved in membrane binding.

Ca^{2+}-dependent Proteases

An interesting Ca^{2+}-activated intracellular protease, sometimes called *calpain*, was discovered during the last decade.[134] The ending -pain refers to its relation with other proteolytic enzymes like papain. It may seem dangerous to have a proteolytic enzyme loose inside a cell, and it must have rather specialized functions and be under strict control. The complete primary structure of the calcium protease ($M_r \approx 80{,}000$) in chicken tissues has recently been deduced from the nucleotide sequence of cloned DNA.[135,136] The findings are quite unexpected.

The protein contains four distinct domains. The first and third domains have no clear sequence homologies with known protein sequences, but the second domain has a high homology with the proteolytic enzyme papain, and the fourth domain is highly homologous to calmodulin. This fourth domain thus has four EF-hand-type Ca^{2+}-binding sites, although the third site has a somewhat unusual loop sequence. Here we apparently are faced with an unusual invention by Nature: by fusing the gene for a protease with that of the canonical Ca^{2+} receptor, she has created a molecule in which a regulatory protein is covalently linked to its target enzyme!

Protein Kinase C

Before we leave our brief survey of intracellular Ca^{2+}-binding proteins, we must write a few lines about an important Ca^{2+}-regulated kinase (a phosphorylating enzyme), i.e., *protein kinase C* (PKC). The activity of this enzyme, or rather family of enzymes,[137] appears to be regulated by three factors: phospholipids, in particular phosphatidylserine; diacyl-glycerols, one of the products of inositol lipid breakdown; and Ca^{2+} ions. The high-activity form of PKC, which appears responsible for much of the phosphorylation activity of many cells, is presumably membrane-bound, whereas the low-activity form may be partly cytosolic (Figure 3.27). The schematic structure of rabbit PKC ($M_r \approx 77$ kDa) according to Ohno *et al.*[138] is shown in Figure 3.28. The Ca^{2+} site(s) are presumably in the regulatory domain. No typical "EF-hand" pattern has been found in the amino-acid sequence. A protein kinase that requires Ca^{2+} but not phospholipids nor calmodulin for activity has been purified from soybean. From the amino-acid sequence the protein appears to have a calmodulin-like Ca^{2+}-binding domain, very much as in calpain.[139]

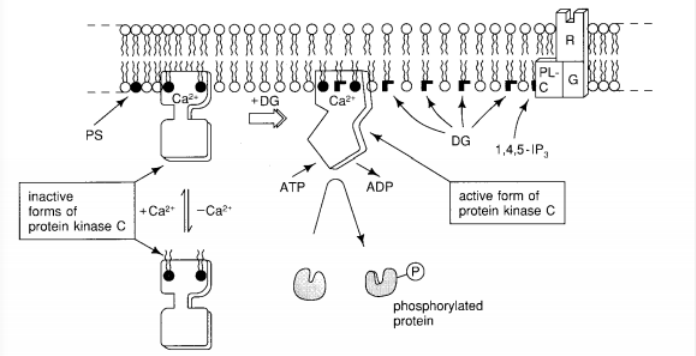

Figure 3.27 - Outline of the cellular events that result in the activation of protein kinase C (PKC). The enzyme apparently exists in at least two states. Recent sequence work indicates that it has a Ca^{2+}-binding site of the EF-hand type. When no Ca^{2+} ion is bound, and when the "concentration" of diacylglycerol (DG) in the inner layer of the plasma membrane is low, the kinase exists in a low-activity form, possibly dissociated from the membrane. When a hormone binds to a plasmamembrane receptor (R), cleavage of phosphoinositol into 1,4,5-IP_3 and DG is induced. The latter lipid may bind to and activate the calcium-loaded form of PKC. The active form of protein kinase C will now phosphorylate other cytoplasmic proteins, and in this way modify their biochemical properties. R = receptor; PL-C = phospholipase C; G = a GTP-binding protein that is assumed to act as an intermediary between the receptor and the membrane bound PL-C.

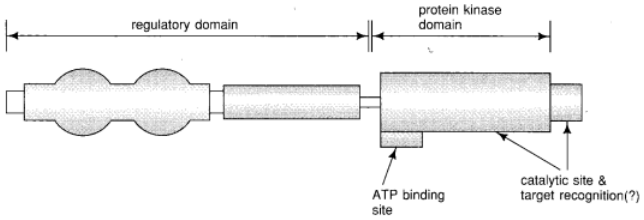

Figure 3.28 - Schematic representation of the structure of rabbit protein kinase C.[138] Three highly homologous protein kinases C were actually identified with $M_r \approx 76{,}800$. The kinase region shows clear similarity with other kinases. The regulatory domain should contain binding sites for Ca^{2+}, phosphatidyl serine (PS), and diacylglycerol (DG).
