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Introduction

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Fifty years have now elapsed since Dean (1941) first formulated the concept of ion pumps; since then the idea has grown into a large and very productive new field of science, in which progress, initially focused on mechanistic and biophysical approaches, is now exponentially growing along structural-molecular lines. In reviewing the field four years ago, Pedersen and Carafoli (1987a,b) had divided the ATP-powered pumps (ion-motive ATPases) into three classes, the P, F, and V types. At that time it seemed safe to predict that the area would be satisfactorily covered, at least for a reasonable span of time, by this subdivision. Contrary to the prediction, however, the field has rapidly started to grow in new directions and the tripartite classification of Pedersen and Carafoli will probably soon have to be further expanded to include new ATPase types—one only need to think of the vast number of nuclear ATPases now being described, of the ATP-powered systems for transporting complex molecules across plasma membranes, or of the prokaryotic ATP-driven pumps for anions or unusual cations. However, even if the future of ion-motive ATPases is rapidly becoming more complex, the F, P, and V types are the only ones on which a sufficient amount of information, functional and structural, is available. The F-ATPases will be the subject of a special issue of *Journal of Bioenergetics and Biomembranes* (Vol. 24, No. 5, 1992) and one of the future issues of the Journal will cover the V-type pumps. This special issue is devoted to the P-type pumps and collects a series of contributions that offer a reasonably complete panorama of the field at its present state of development. The contributions deal with both mechanistic and structural aspects; in keeping with modern trends and to privilege the areas in which the most exciting developments are now occurring, the latter have taken primacy. One of the pumps is discussed in two, rather than one, separate articles: the Na^+/K^+-ATPase. This essentially reflects historical aspects, i.e., the fact that this pump has been studied at greater length and more extensively than the others, and has, in a sense, served as a paradigm for the studies on all other pumps of the class. The contribution will then cover the Ca^{2+}-ATPase of sarco (endo)-plasmic reticulum, the H^+ ATPase of the plasma membrane, the Ca^{2+}-ATPase of the plasma membrane, the H^+/K^+-ATPase, and the newly discovered prokaryotic Mg^{2+}-transporting ATPase. Unexpected technical difficulties have prevented inclusion of the K^+-transporting ATPase of *E. coli* (Hesse *et al.*, 1984). This ATPase has unusual properties, since it apparently consists of several subunits of which one has the catalytic function, while the others all appear to play some sort of regulatory role.

The aim of this brief introduction is to offer a general background to the contents of the seven contributions contained in the issue, to point toward the outstanding problems, and, possibly to indicate guidelines for further research and developments. One important point, apart from the obvious common aspects of the aspartyl-phosphate intermediate and of the vanadate inhibition, is the similar membrane architecture of all these pumps: the common pattern is that of a relatively minor membrane-intrinsic portion, and of the asymmetric protrusion of most of the pump mass from the membrane environment. These pumps protrude into the cytosol and do so with two main units: Fig. 1, which shows the Ca^{2+} pump of the plasma membrane (Carafoli, 1991), embodies the architectural aspects common to all pumps of the P-class. Although the essential features of the model can be considered as conclusively ascertained, it is well to remember that some of its aspects, even important aspects, are still hypothetical. For instance, the number of transmembrane domains, which varies in any case from pump to pump, is still a subject of discussion. By contrast, whether their number is odd or

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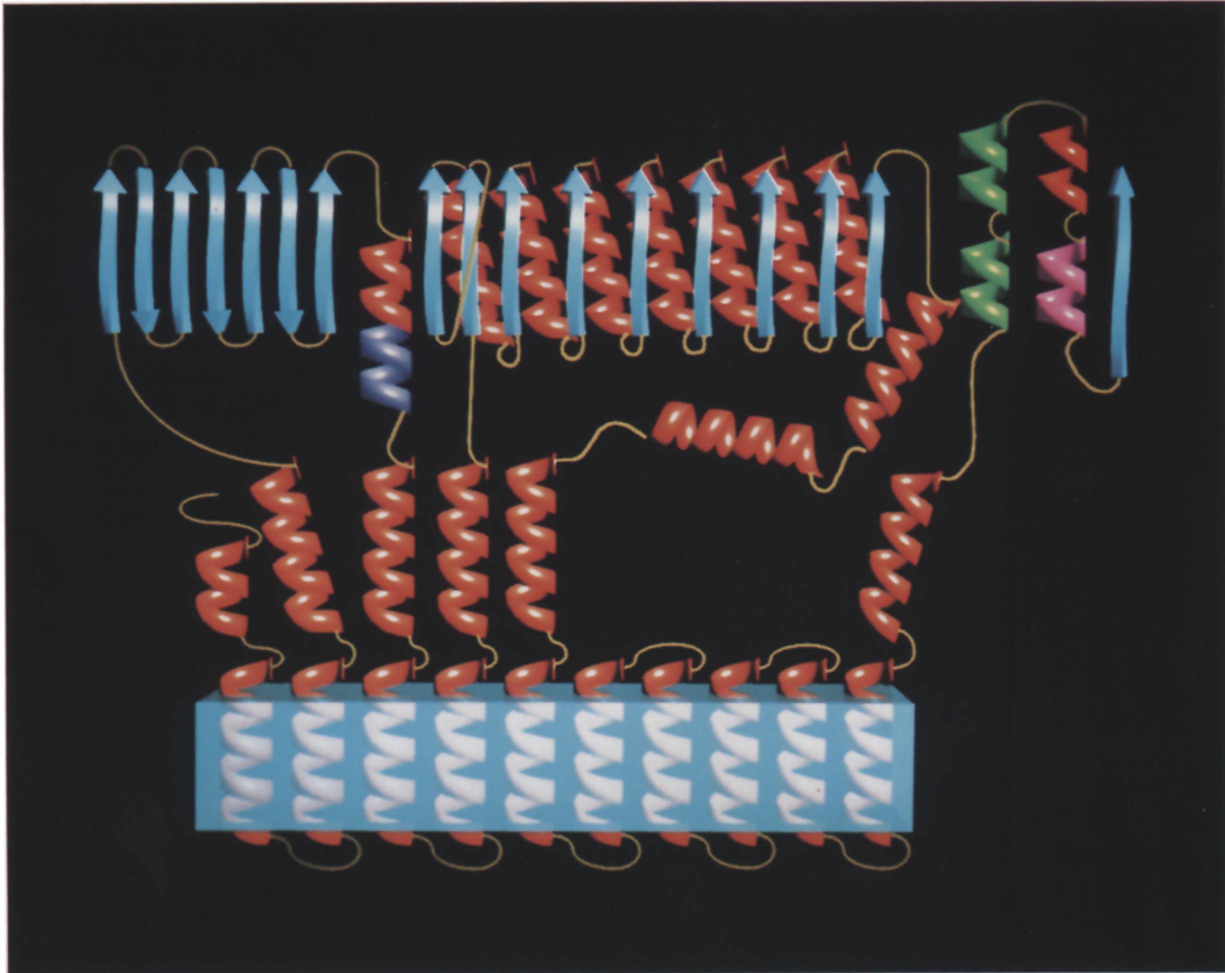

Fig. 1. The membrane architecture of the plasma membrane Ca^{2+} pump. The pump organization in the membrane is similar to that of all other pumps of the P-class. The secondary structure predictions are taken from the University of Wisconsin Genetics Computer Group sequence analysis software package, taking into account the alignment of other P-type ATPases (Taylor and Thornton, 1984; Green, 1989). Spiral forms indicate α helices, arrows β sheets. Color code: green, calmodulin-binding domain, subdivided into subdomains; purple, domain containing the substrate site for protein kinase A; dark blue, putative phosolipid-responsive domain (the Ca pump of the plasma membrane is stimulated by acidic phospholipids).

even, a problem which led to some conflicting conclusions in recent years (Ovchinnikov *et al.*, 1988), is no longer being discussed. By general consensus, it has now been agreed that both termini of all these pumps are on the same side of the membrane, i.e., the number of transmembrane domains is by necessity even. However, the topographical scheme of Fig. 1, i.e., the actual number of the transmembrane domains, has been experimentally verified only in some limited portions and only on some of the ATPases (Clark *et al.*, 1990; Mattews *et al.*, 1990; Sachs *et al.*, 1989; Feschenko *et al.*, 1992).

The short N-terminal segment of the pumps is predicted to be essentially α-helical. Of the two main protruding units, the first from the N-terminus has been termed, based on work on the sarcoplasmic reticulum ATPase, the transducing unit, i.e., a domain that would somehow couple the hydrolysis of ATP to the translocation of the ions and which can be predicted to essentially consist of antiparallel β-strands (Taylor and Thornton, 1984; MacLennan *et al.*, 1985). The second extramembrane domain, which is the largest, contains the site of aspartyl phosphate formation, the ATP-binding site, and a flexible hinge formed by a pair of helices. The secondary structure of the large domain is predicted to predominantly consist of alternating α-helices and parallel β-strands. The C-terminal portion of the pumps, which is generally

short, is instead very long (> 150 residues) in the Ca^{2+} pump of the plasma membrane (Fig. 1). The C-terminal domain of this pump consists of large α-helical portions and contains important regulatory domains, i.e., the calmodulin-binding domain (James *et al.*, 1989) and the substrate site for kinase-mediated phosphorylations (James *et al.*, 1989; Wang *et al.*, 1991). The P-type pumps are thus essentially intracytoplasmic enzymes, anchored to the membrane by an even number of intrinsic segments: the loops connecting these segments on the external side (i.e., the non-cytoplasmic side) of the membrane share the property of being short and devoid of known important physiological functions. However, one prominent exception, pharmacological if not physiological, is the presence of the ouabain binding site on the external loops of the Na^+/K^+-ATPase.

One prominent problem, which is still open in all these ATPases, is that of the location of the catalytic cationic binding site. Site-directed mutagenesis work (Clarke *et al.*, 1990) on the sarcoplasmic reticulum calcium ATPase suggests a location within the transmembrane sectors: the site would be contributed by four of the transmembrane helices, possibly the fourth, fifth, sixth, and eighth. Clearly, site-directed mutagenesis is a powerful tool to shed light on this important problem: as the *in vitro* expression of these pumps will become more and more routine, one can expect the rapid development of important information on this (and other) issue(s).

The Na^+/K^+-ATPase is composed of a catalytic subunit α and of an accessory subunit β, whose function as yet has not been established. A similar situation seems to prevail in the case of the H^+/K^+-ATPase, and it is tempting to suggest that the accesory subunit, while not involved in the catalytic cycle proper, may serve some regulatory function. In this connection, it is very interesting that the longest of the pumps of this class, the Ca^{2+} pump of the plasma membrane, has a large C-terminal protruding domain which contains several regulatory sites (see above). The calcium pump of the sarcoplasmic (endo) reticulum, while not having a covalently bound regulatory domain, has an "accessory" regulatory domain, i.e., the membrane-intrinsic protein phospholamban. Recent work has pointed toward several similarities between the regulation features of phospholamban on the Ca^{2+} pump of sarco (endo) plasmic reticulum and of the C-terminal calmodulin-binding domain of the Ca^{2+} pump of the plasma membrane (Chiesi *et al.*, 1991). One could thus cautiously suggest the general possibility of regulatory domains of these pumps either covalently bound or in the form of a separated protein.

Several other points of general significance could be touched upon in this Introduction–for example, the puzzling observation that the prokaryotic Mg^{2+}-transporting ATPase apparently moves Mg^{2+} down its electrochemical gradient—but at this point, in the interest of space, it would be better to go on with the reading of the contributions. It is hoped that this special issue will raise interest in these very interesting enzymes and will help to solve some of the outstanding problems.

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