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STRUCTURE AND FUNCTION OF V-ATPases IN ENDOCYTIC AND SECRETORY ORGANELLES

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Summary

Chromaffin granules and clathrin-coated vesicles are major sources for V-ATPases of mammalian cells. Studies of these organelles have helped us to understand the structure and function of the enzyme. It was shown that V-ATPases are composed of distinct catalytic and membrane sectors containing several subunits. The subunit stoichiometry was determined to be 3*A*, 3*B*, 1*C*, 1*D*, 1*E*, 6*c* (proteolipids), 1Ac115 and ?Ac39. Additional subunits are likely to be discovered. Resolution and reconstitution of the enzyme revealed that the catalytic and membrane sectors are interdependent for their partial activity. The catalytic sector has no ATPase activity when detached from the membrane sector, and the membrane sector when depleted of the catalytic sector does not conduct protons. The mechanistic significance of these properties is discussed.

Subunit structure

The vacuolar H^+-ATPase (V-ATPase) of chromaffin granules and clathrin-coated vesicles has been studied more extensively than all other organelles of mammalian cells. Historically, chromaffin granules provided the first evidence for the existence of an H^+-ATPase in the vacuolar system of eukaryotic cells (Kirshner, 1962; Bashford *et al.* 1975; Njus and Radda, 1978; Mellman *et al.* 1986). It was demonstrated that catecholamine uptake is driven by the proton-motive force generated by an ATP-dependent proton pump, later named V-ATPase (Pollard *et al.* 1976; Johnson and Scarpa, 1976; Casey *et al.* 1977; Holz, 1978, 1979; Schuldiner *et al.* 1978). Therefore it was established that an electrochemical gradient of protons generated by an H^+-ATPase is the driving force for catecholamine uptake (Cidon and Nelson, 1983), a process that is a fine example of the chemiosmotic theory (Mitchell, 1968). Lysosomes were next in providing evidence that an ATP-dependent proton pump generates and maintains their acidic interior (Schneider, 1979, 1981; Reeves and Reames, 1981). Clathrin-coated vesicles were added to the family when ATP-dependent proton accumulation into the organelles was clearly demonstrated in two laboratories (Forgac *et al.* 1983; Stone *et al.* 1983). Subsequent structural studies demonstrated that the V-ATPases of the two different organelles are almost identical in their subunit structure and biochemical properties (Xie and Stone, 1986; Arai *et al.* 1988; Nelson, 1989; Moriyama and Nelson, 1989*a*). It was shown that the catalytic sector (V_1) is composed of five polypeptides, denoted as subunits *A* to *E* (Nelson, 1989). This sector can be dissociated from the membrane by treatment with urea

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(Xie and Stone, 1986), KI plus MgATP (Arai *et al.* 1989) or by cold inactivation in the presence of NaCl and MgATP (Moriyama and Nelson, 1989*a*,*b*,*c*). The genes encoding subunits *A, B, C* and *E* were cloned and sequenced (Hirsch *et al.* 1988; Südhof *et al.* 1989; Nelson *et al.* 1990; Pan *et al.* 1991; Puopolo *et al.* 1991, 1992). The membrane sector (V_o) is composed of more than four subunits with relative molecular masses of 115, 32, 20 and 16×10^3 (Arai *et al.* 1988; Moriyama and Nelson, 1989*a*). The genes encoding three of these subunits were cloned and sequenced (Mandel *et al.* 1988; Wang *et al.* 1989; Perin *et al.* 1991), and only the gene of the alleged subunit of 20×10^3 M_r awaits cloning. Table 1 depicts the subunit composition reported for V-ATPases from different sources. There is general agreement on the presence of subunits *A, B, C* and *E* of V_1 and the proteolipid of V_o in all of the preparations reported so far. The 115×10^3 M_r polypeptide is probably present in all mammalian V-ATPases and is present in yeast and *Neurospora* cells. In plants, however, it is not clear if it is necessary for the activity of the enzyme (Ward *et al.* 1992). The 39×10^3 M_r polypeptide was shown to be present only in mammalian V-ATPases. Several additional polypeptides were identified in preparations of V-ATPases from various sources, but their involvement as subunits of the enzyme was not proven (Percy and Apps, 1986; Xie and Stone, 1986; Gluck and Caldwell, 1987).

The amino acid sequences of V-ATPase subunits revealed their relationships to F-ATPase subunits (Nelson, 1989). The sensitivity of eukaryotic V-ATPases to –SH reagents was localized to cysteine 254 in subunit *A* of the bovine enzyme (Feng and Forgac, 1992). Reconstitution studies together with chemical modifications shed some light on the function of the various subunits in the catalytic activity of V-ATPases. Nevertheless, we are far from understanding the fine structure of the enzyme and its mechanism of action.

Function of V-ATPases in the various organelles

Whereas the function of the V-ATPase in coated vesicles is to provide limited acidification for processes such as recycling of receptors, the main function of the enzyme in synaptic vesicles and secretory granules is to provide energy for a massive uptake of

Table 1. *Subunit structure of mammalian V-ATPases*

	V_1 sector					V_o sector				
Source of V-ATPase	*A*	*B*	*C*	*D*	*E*	Ac115	Ac39	*a*	*c* (proteolipid)	Additional
Clathrin-coated vesicles	68*	57*	40	34	33	96*	38	20	16	
Chromaffin granules	68*	57	44*	34	33	115	32*	20	16*	
Kidney	70	56	42	33	26*		38		15	45, 14, 12
Lysosomes	72	57	41	34	33		39	18	15	55
Golgi apparatus	68	58	40	34	33		37		16	

Apparent relative molecular masses of the various subunits are given $\times10^{-3}$.

*Relative molecular masses deduced from cDNA sequences (Puopolo *et al.* 1991, 1992; Perin *et al.* 1991; Pan *et al.* 1991; Nelson *et al.* 1990; Wang *et al.* 1989; Mandel *et al.* 1988; Hirsch *et al.* 1988). The remaining relative molecular masses were estimated on SDS gels (Xie and Stone, 1986; Moriyama and Nelson, 1989*a*,*b*,*c*; Wang and Gluck, 1990; Young *et al.* 1988).

neurotransmitters (Cidon and Shira, 1989; Moriyama *et al.* 1990). Synaptic vesicles contain up to $0.8\,mol\,l^{-1}$ of neurotransmitters, and chromaffin granules may contain $0.5\,mol\,l^{-1}$ catecholamines as well as $0.15\,mol\,l^{-1}$ ATP. In addition to these chemicals, chromaffin granules contain a large number of enzymes and proteins sensitive to low pH. Therefore, the V-ATPase should provide abundant energy in the form of proton-motive force while preventing overacidification in the interior of the organelle (Moriyama and Nelson, 1988). Understanding the mechanism controlling these processes is one of the most challenging tasks in the field. It will also be interesting to learn whether the same enzyme functions in the wide variety of synaptic vesicles in the brain. V-ATPases are also likely to be involved in the biogenesis of various internal organelles in brain cells. The initial studies of V-ATPases in chromaffin granules and clathrin-coated vesicles provided the foundation for such studies.

Future

In the future more attention will be given to the specific properties of V-ATPases in the various organelles. One of the most challenging questions is how such a conserved enzyme can function in so many organelles each with specific requirements for the extent and composition of the proton-motive force. It was observed that only a single gene encodes the *A* subunit in the bovine genome (Puopolo *et al.* 1991). Even the presence of isogenes encoding subunits *B* and *E* cannot explain the diversity of V-ATPases in eukaryotes (Puopolo *et al.* 1992). Moreover, it is not clear how the different gene products can assemble specific enzymes in the different organelles because no signal sequences have yet been identified. Recently, it became apparent that V-ATPases function not only in internal organelles but also on the plasma membrane (Nelson, 1991). A V-ATPase with unique enzymatic properties was recently identified in osteoclasts (Chatterjee *et al.* 1992). One of the ways to study specific mammalian gene products is to express them in yeast mutants in which the corresponding gene has been interrupted (Beltrán *et al.* 1992). A combination of yeast genetics with biochemistry in mammalian cells is a powerful approach that is likely to advance us to the future.

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