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The Structure of the Vacuolar ATPase in *Neurospora crassa*

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The filamentous fungus *Neurospora crassa* contains many small vacuoles. These organelles contain high concentrations of polyphosphates and basic amino acids, such as arginine and ornithine. Because of their size and density, the vacuoles can be separated from other organelles in the cell. The ATP-driven proton pump in the vacuolar membrane is a typical V-type ATPase. We examined the size and structure of this enzyme using radiation inactivation and electron microscopy. The vacuolar ATPase is a large and complex enzyme, which appears to contain at least thirteen different types of subunits. We have characterized the genes that encode eleven of these subunits. In this review, we discuss the possible function and structure of these subunits.

KEY WORDS: ATPase; V-ATPase; vacuolar ATPase; vacuole; fungi; *Neurospora*; proton pump.

INTRODUCTION

Neurospora crassa is a filamentous fungus that grows vigorously in simple media and is amenable to genetic manipulation. The "one gene–one enzyme" hypothesis was developed from experiments done with *N. crassa* in the 1940s (Beadle and Tatum, 1941). The organism has served as a useful experimental system for the genetic analysis of metabolic pathways and for investigations into the regulation of gene activity (Perkins, 1992). In addition, *N. crassa* has been an ideal organism for investigation of bioenergetic and transport phenomena in eukaryotic cells. Liquid cultures of *N. crassa* are a rich source of mitochondria, and isolated preparations retain a tight coupling between oxygen consumption and ATP synthesis. When grown on solid medium the filamentous hyphae are large with a rigid wall that can be penetrated by microelectrodes, allowing direct measurements of electrical potential gradients, ion currents, and pH. The types of experiments that can be done with *N. crassa* have permitted a close examination of the major ATP-driven proton pumps: the F-type ATPase in the mitochondria, the P-type ATPase in the plasma membrane, and the V-type ATPase in the vacuole (Bowman and Bowman, 1996; Rao and Slayman, 1996).

THE VACUOLE IN *N. crassa*

Our investigation of the vacuolar ATPase began, in part, because methods had been developed to isolate vacuoles from *N. crassa* in good yield and purity (Cramer *et al.*, 1983; Bowman and Bowman, 1988). The size and shape of the vacuole can vary dramatically in different growth conditions. In rapidly growing cultures the vacuoles are small, similar in size and shape to lysosomes of animal cells. They accumulate high concentrations of arginine, ornithine, histidine, and polyphosphate (Cramer and Davis, 1984). When centrifuged through sucrose gradients, the vacuoles migrate as very dense particles, which can be separated from mitochondria and other organelles (Bowman and Bowman, 1982; Cramer *et al.*, 1983).

The vacuoles are not merely storage compartments. They play a dynamic role in the regulation of amino acid metabolism (Bowman and Davis, 1977a,b; Davis, 1986). As has been demonstrated in *Saccharomyces cerevisiae*, the vacuole is also a major site for the sequestration of calcium and plays a dynamic role

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in regulating the calcium concentration of the cytosol (Miller *et al.*, 1990; Tanida *et al.*, 1995).

Like animal cell lysosomes the vacuoles in *N. crassa* are acidic compartments and contain a variety of hydrolytic enzymes. We have characterized one of the major vacuolar proteases, the product of the *pep-4* gene, and have generated strains in which this gene is inactivated (Vazquez-Laslop *et al.*, 1996). We have also identified the gene that encodes the *N. crassa* homolog of carboxypeptidase Y (I. E. Hunt and B. J. Bowman, unpublished).

The V-type ATPase plays a central role in the function of the vacuole. It generates an electrochemical gradient for protons that drives the transport of molecules across the vacuolar membrane. It also acidifies the interior of the vacuole to provide the correct chemical environment for maturation and function of hydrolytic enzymes. We have characterized the kinetic behavior, substrate requirements, and effect of inhibitors on the vacuolar ATPase, information that is summarized in recent reviews (Bowman *et al.*, 1992; Bowman and Bowman, 1996). In this report we wish to focus on the structure and subunit composition of the vacuolar ATPase.

ANALYSIS OF STRUCTURE BY RADIATION INACTIVATION AND ELECTRON MICROSCOPY

Radiation inactivation data provided an early estimate of the size of the vacuolar ATPase in *N. crassa*. The results indicated that the vacuolar ATPase was 520 kDa, while the homologous mitochondrial ATPase, measured in the same experiment, was 460 kDa (Bowman *et al.*, 1986). It is interesting to look at these data in the light of current estimates of the size of these enzymes. For the mitochondrial ATPase, the radiation inactivation data are in good agreement (82%) with the molecular weight obtained from the known sequences of subunits (560 kDa). For the vacuolar ATPase, the size estimate by radiation inactivation is only 62% of the total molecular weight of the known subunits (830 kDa). Although the discrepancy may be due to an inherent inaccuracy in the method, it is also possible that the data point to a difference between the V- and F-type ATPases. The method of radiation inactivation measures the "functional size" of an enzyme. Some of the large subunits of the V-type ATPase, which have no obvious homologs in the F-type ATPase (e.g., subunits C and H, see below), may not be essential for ATPase activity.

Like the mitochondrial ATPase, the vacuolar ATPase has a ball and stalk structure when negatively stained and viewed in the electron microscope (Bowman *et al.*, 1989; Dschida and Bowman, 1992). However, the vacuolar ATPase is significantly different in size and shape from the mitochondrial ATPase. The diameter of the F_1 sector of the mitochondrial ATPase estimated from our electron micrographs agrees precisely with data obtained from X-ray crystallography of the bovine F_1-ATPase (Abrahams *et al.*, 1994). Thus, differences seen in the vacuolar ATPase are unlikely to be just artifacts of microscopy. Assuming that the ATP-binding subunits (A and B) of the enzyme fold in the same way as the α and β subunits of the bovine mitochondrial enzyme, then the extra mass in the vacuolar ATPase must be due to extra subunits, larger subunits, or both. The V_1 sector is approximately 20 A wider than mitochondrial F_1. Part of the difference is probably due to the larger size of the A subunits (67 kDa) compared to the homologous β subunits (51 kDa) (Bowman *et al.*, 1988; Bowman and Knock, 1992). However, to account for the observed difference, it seems likely that some of the "extra" subunits found in the vacuolar ATPase (e.g., C, D, E, or H) are part of the head region of V_1.

The stalk of the vacuolar ATPase appears to be 20 A longer and 5 A wider than in the mitochondrial ATPase (Dschida and Bowman, 1992). Few data are available to address the question of which subunits form the stalk. In F-type ATPases it seems clear that the γ subunit extends the entire length of the stalk and contacts the V_0 sector embedded in the membrane (reviewed in Fillingame, 1996, and Nakamoto, 1996). In the vacuolar ATPase, two subunits, D and E, are likely candidates to play the same role as the γ subunit in F_1. The question of whether subunit D or E is more likely to be a γ homolog is addressed further below.

Several laboratories have recently reported a structure resembling a second stalk, visible in electron micrographs of both F-type and V-type ATPases (Boekema *et al.*, 1997, 1998; Wilkens and Capaldi, 1998). In our images of the *N. crassa* vacuolar ATPase, a second stalk was not obvious, but extra protrusions from the base of the stalk were consistently seen. We cannot yet identify the polypeptides associated with these protrusions.

SUBUNIT COMPOSITION OF THE VACUOLAR ATPase

A major goal of our laboratory has been to determine the complete subunit composition of the vacuolar

Table I. Subunits of the *Neurospora crassa* Vacuolar ATPase[a]

	Name	Mol. wt. (kDa)	Gene	F-ATPase homolog
V_1	A	67	*vma-1*	β
	B	57	*vma-2*	α
	H	50	*vma-13*	
	C	47	Not identified	
	D	28	*vma-8*	γ?
	E	26	*vma-4*	γ?
	F	13	*vma-7*	δ?
	G	13	*vma-10*	b
V_0	a	98	*vph-1*	a?
	d	41	*vma-6*	
	c	16	*vma-3*	c
	c′	16	*vma-11*	c
	c″	?	Not identified	c

[a] We have adopted the nomeclature of Stevens and Forgac (1998). All molecular weights are derived from the sequences of the genes except for subunit C, which was determined by gel electrophoresis as shown in Fig. 2.

ATPase. Comparison of data from many laboratories suggests that the subunit composition of the V-type ATPase is remarkably conserved. At present the highest level of subunit complexity has been reported for *S. cerevisiae*; thirteen different subunits are required as structural components of the functional enzyme (Stevens and Forgac, 1997). As shown in Table I and Fig. 1, we have isolated homologs to eleven of these thirteen subunits. (Note: in this article we adhere to the original meaning of the word "homologous," i.e., evolved from a common ancestor.) Subunits C and c″ remain to be found. V-type ATPases from mammalian cells appear to be composed of the same set of subunits, although the precise description of the proteolipid components (c, c′, c″) has not yet been completed (Stevens and Forgac, 1997). We have no evidence for unique subunits in the *N. crassa* enzyme.

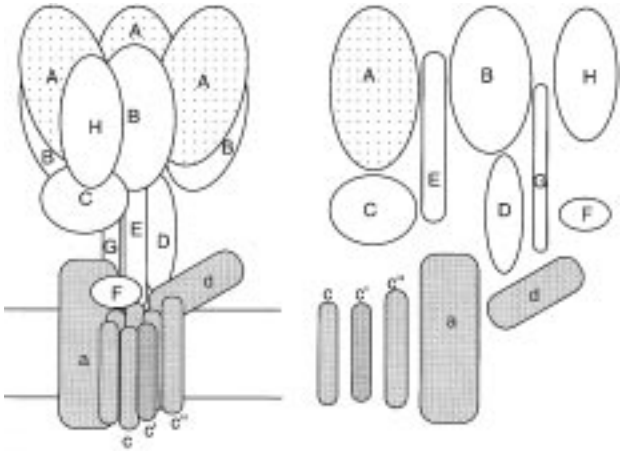

Fig. 1. Model of the *N. crassa* vacuolar ATPase. The size of each subunit and the name of the corresponding gene is given in Table I. The dark-filled subunits are in the membrane-associated V_0 sector, while the others are in the peripheral V_1 sector.

The *N. crassa* enzyme can be purified by solubilization of vacuolar membranes in detergent (e.g., 0.4% Zwittergent 3–14) followed by centrifugation through a sucrose gradient (Bowman *et al.*, 1986). In one set of experiments, the preparation was subjected to further fractionation on a Bio-Gel A-0.5 m column. At least eleven different polypeptides copurified with ATPase activity (Fig. 2). The preparation contained polypep-

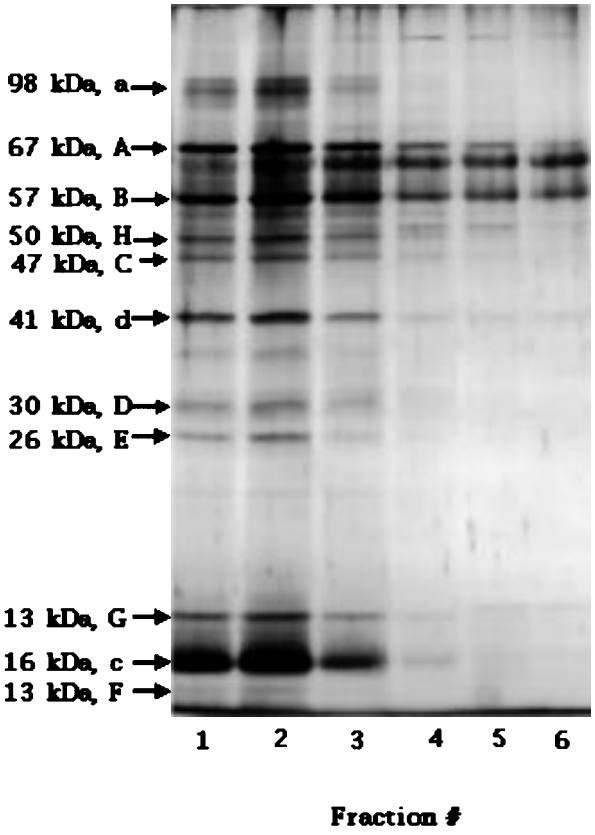

Fig. 2. Polypeptide composition of the *N. crassa* vacuolar ATPase. Vacuolar membranes (2 mg protein per ml) were washed in 0.1% dodecyl maltoside, pelleted, and resuspended in the same volume of 0.2% Zwittergent 3–14. This detergent-solubilized enzyme was layered onto a 10% glycerol gradient and centrifuged at 277,000 × *g* for 16 h. The ATPase, found in the top fraction, was then loaded on a Bio Gel A-0.5 m column. Essentially all of the protein emerged in six fractions, which were analyzed by gel electrophoresis, as shown in the figure. The peak of ATPase activity was in fractions 2 and 3. The molecular weight of each band was determined by comparison to molecular weight standards run in the same polyacrylamide gel.

tides corresponding to all thirteen putative subunits, assuming the three proteolipids migrated as a single band. Most of our preparations of the vacuolar ATPase do not appear to have the complete complement of polypeptides. Subunits C and D are usually not visible and subunits a and B are highly susceptible to proteolysis. The specific ATPase activities of the best preparations are 2–4 μmol/min/mg protein, the same as the specific activity of purified vacuolar membranes (Bowman *et al.*, 1986, 1992). We suspect that some subunits of the vacuolar ATPase from *N. crassa* easily dissociate from the enzyme, resulting in a loss of activity.

FUNCTION OF THE V_1 SUBUNITS

Subunits A and B

Data obtained from *N. crassa* and from the plant *Daucus carota* led to the initial discovery that vacuolar ATPases are related to F-type ATPases (B.J. Bowman *et al.*, 1988; E. J. Bowman *et al.*, 1988; Zimniak *et al.*, 1988). Subunits A and B are the homologs of the β and α subunits, respectively, in the F-type ATPase. These subunits bind nucleotides and form the sites of ATP hydrolysis and synthesis. Analysis of the gene for the A subunit revealed an interesting feature, the significance of which has not yet been determined. Subunit A contains a region of approximately 90 residues (~10 kDa), which has no counterpart in the β subunit of F_1 (Zimniak *et al.*, 1988; Gogarten *et al.*, 1989). By using sequence alignments of the A and β subunits and the atomic structure of F_1 (Abrahams *et al.*, 1994), we can determine the likely position of this extra nonhomologous domain. Its predicted position is on the top of the V_1 sector, toward the periphery, roughly in the same region where the δ subunit is postulated to be situated in the F-type ATPase (Ogilvie *et al.*, 1997).

Subunits H and C

The next two largest subunits, named H (54 kDa) and C (42 kDa) have no obvious counterparts in the F-type ATPase (Stevens and Forgac, 1997). In partially purified vacuolar ATPase from *N. crassa*, we often see two polypeptides of approximately 54 kDa. We have used gel electrophoresis to purify both polypeptides and have digested them with trypsin. The pattern of tryptic peptides obtained is similar, but not identical, suggesting the two polypeptides are slightly modified versions of the same gene product (K. Tenney and B. Bowman, unpublished). A similar result was reported for the V-type ATPase from bovine-coated vesicles (Zhou *et. al.*, 1998). For the bovine enzyme, the two polypeptides were shown to be derived from a single gene, possibly by alternative splicing. The gene appears to be the homolog of *VMA13* in *S. cerevisiae* (Ho *et al.*, 1993). We have recently isolated the *vma-13* homolog in *N. crassa* and are now characterizing it.

We have little information concerning subunit C in *N. crassa*. Some purified preparations contain a polypeptide similar in size to the C subunit observed in other V-type ATPases (Fig. 2). In many preparations, however, this polypeptide is not visible (e.g., Fig. 2 in Bowman *et al.*, 1992). We have not yet identified the gene that encodes this subunit in *N. crassa*.

Subunits D and E

One of the most important questions regarding the V-type ATPase is whether the enzyme has a homolog to the γ subunit of the F-type ATPase. A prediction from comparative analyses of the sequences of the A and B subunits and of the β and α subunits of the F-type ATPase is that the two enzyme complexes are likely to form similar tertiary and quaternary structures. The X-ray crystal structure of bovine F_1-ATPase revealed two helical segments of the γ subunit (the N and C termini of the polypeptide, Fig. 3) fitting tightly inside the cavity formed by the α and β subunits (Abrahams *et al.*, 1994). Therefore, it seems probable that some polypeptide in the V-ATPase also forms two helical segments that fit within the cavity formed by the A and B subunits. The D and E subunits are the best candidates to play this role. Both are approximately the correct size and predicted to be predominantly helical in structure (Bowman *et al.*, 1995; Nelson *et al.*, 1995). Furthermore, experiments designed to investigate the assembly of the V-type ATPase in *S. cerevisiae* indicated that either the D or E subunits, or both, might function as the γ homolog (Tomashek *et al.*, 1997).

Earlier we characterized the *vma-4* gene, which encodes the E subunit in the *N. crassa* vacuolar ATPase (Bowman *et al.*, 1995). The gene product is a 25, 746-dalton protein that is a prominent component in purified preparations of the vacuolar ATPase. Recently, we have characterized the gene encoding subunit D,

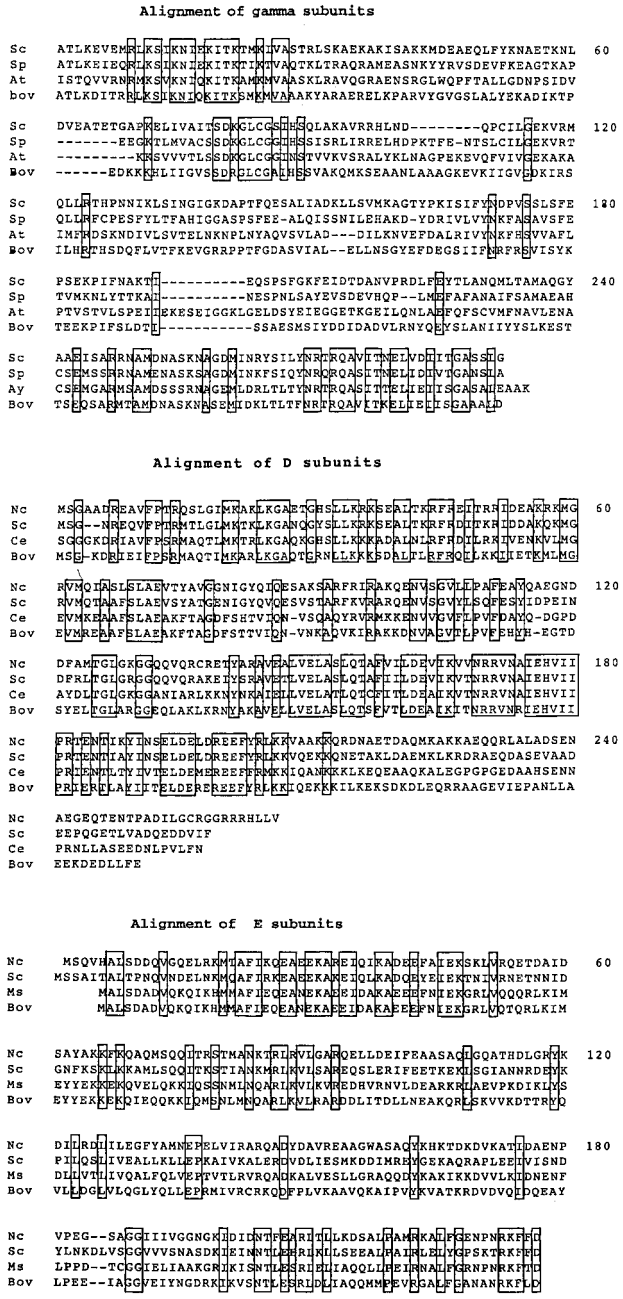

Fig. 3. Comparison of the amino acid sequences of the γ subunit of F-type ATPases with the D and E subunits of V-type ATPases. All sequences were obtained from GenBank. Regions of sequence identity are boxed. For each subunit, four organisms were selected in which the sequences show significant evolutionary divergence. The genes encoding all three subunits have been identified only in *Saccharomyces cerevisiae* (Sc) and *Bos taurus* (Bov). The other organisms used are *Neurospora crassa* (Nc), *Caenorhabditis elegans* (Ce), *Saccharomyces pombe* (Sp), *Arabidopsis thaliana* (At), and *Manduca sexta* (Ms).

a polypeptide that is usually present in low amounts in our purified enzyme preparations. The gene, named *vma-8*, contains 3 introns and maps to linkage group IVR, near the *cot-1* locus (see GenBank accession No. AF053230). The predicted gene product has 266 amino acids and a molecular weight of 29,732 daltons. Except for the C-terminus the D subunit is highly conserved. The sequence of the *N. crassa* protein is 65% identical to that of *S. cerevisiae* and 48% identical to subunit D in bovine cells.

Whether subunit D or E is the more likely homolog of γ is difficult to say. Previously, we suggested that subunit E may play this role (Bowman *et al.*, 1995). In *N. crassa* subunit E is tightly associated with the enzyme, whereas the D subunit appears to bo easily lost. We also noted that in reconstitution of the bovine V-type ATPase, the E subunit is required for ATPase activity while the D subunit is not (Crider *et al.*, 1997). Figure 3 shows a comparison of the amino acid sequences of the γ subunit from F-type ATPases and the D and E subunits from V-type ATPases. In the overall pattern of sequence conservation, the E subunit more closely resembles the γ subunit. Both have conserved regions at the N- and C-termini and the lengths of the C-termini are similar. By contrast, the D subunits are highly variable in their C-terminal regions. Another characteristic property of γ subunits from different organisms is a pronounced basic pI value, 8.9–9.3. In this property, D subunits resemble γ, but the E subunits have acidic pI's of 5.3 to 5.4.

At this time the arguments for either D or E as the homolog of γ are far from compelling. For example, knowing that the catalytic core of F-type ATPases is highly conserved, we would expect to find conserved residues in the V-type subunit that is the homolog of γ. In the crystal structure of the F-type ATPase, a key site of interaction between the β subunit and the γ subunit has been identified (Abrahams *et al.*, 1994). Three residues in the β subunit, Asp-316, Thr-318, and Asp-319, form hydrogen bonds with Arg-254 and Gln-255 in the γ subunit. The three residues involved in the β subunit are highly conserved within F-type ATPases and are identical in V-type ATPases. This region of the γ subunit is also highly conserved in F-type ATPases (Fig. 3). However, no similar region is found in either the D or E subunits of V-type ATPases. Analysis of the sequences by helical wheel plots also failed to reveal any striking similarities in the position of amino acids.

Furthermore, with regard to the bovine reconstitution experiments it is possible that significant vacuolar

ATPase activity can be obtained with a subcomplex of the enzyme that lacks the γ homolog. Experiments of this type have been reported for the F-type ATPase (Sokolov and Gromet-Elhanan, 1996). Another complexity concerns the length of the stalk in F- and V-type ATPases. Evidence from electron microscopy shows the stalk of the *N. crassa* vacuolar ATPase to be approximately 20 A longer than the stalk of the mitochondrial ATPase (Dschida and Bowman, 1992). There is good evidence that the γ subunit extends the entire length of the stalk and binds to a membrane-embedded proteolipid (reviewed in (Fillingame, 1996). Subunits D and E are both smaller polypeptides than the γ subunits. If either of these is homologous to γ, it must fold in a significantly different way. Alternatively, two polypeptides might play the role of the γ subunit.

Subunit F

We have recently identified the gene in *N. crassa* that encodes subunit F. This gene, named *vma-7*, is located on linkage group 4 near the *met-1* locus. It encodes a protein of 124 residues with a high degree of sequence similarity to F subunit proteins from other organisms (Fig. 4). The gene contains three introns, which interrupt the protein coding region at positions corresponding to amino acids nos. 20, 32, and 78 (see GenBank, accession No. AF099136).

The sequence of the F subunit does not seem to have retained any similarity to sequences of subunits in the F-ATPase. Nelson *et al.*, 1994 proposed that the function of the F subunit may be similar to that of the ε subunit of the F-type ATPase in eubacteria and chloroplasts, linking the V_1 and V_0 sectors. Recent data from studies of the *E. coli* ATPase have provided direct evidence that the ε subunit binds both the polar loop of subunit c and also binds the γ subunit (Garcia and Capaldi, 1998). The homologous protein in mitochondrial ATPases is called the δ subunit. The ε/δ proteins are very similar in size and amino acid composition to the F subunit. For example, the *N. crassa* subunit F is 13,816 kDa with a pI of 5.62, the *E. coli* ε subunit is 14,935 kDa, pI 5.36, and the *N. crassa* δ subunit is 14,675 kDa with a pI of 4.37.

It is perhaps not surprising that the sequence of subunit F is dissimilar to the ε/δ subunits because the latter are poorly conserved within F-type ATPases. For example, comparison of the *E. coli* ε subunit, the corn chloroplast ε subunit, and the *N. crassa* mitochondrial δ subunit shows only 7% sequence identity (Walker *et al.*, 1985). Even comparison among mitochondrial subunits shows a low degree of sequence similarity. The bovine and *N. crassa* δ subunits are 30% identical. By contrast the F subunits of V-ATPases are more highly conserved. Bovine and *N. crassa* sequences are 50% identical. This high degree of sequence conservation suggests that the F subunit binds to a highly conserved region in another polypeptide. If it is really the equivalent of *E. coli* ε, then it likely binds to a polar loop in one of the proteolipid subunits (see below) and to a region of either subunit D or E.

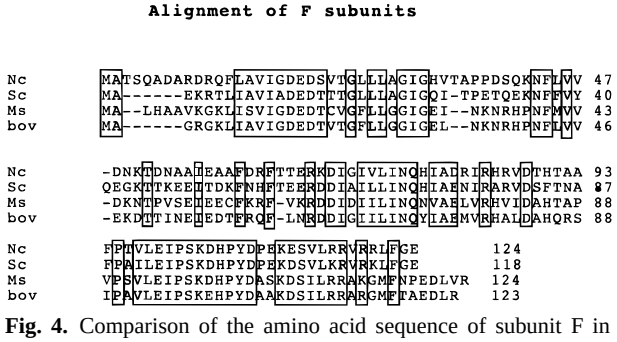

Fig. 4. Comparison of the amino acid sequence of subunit F in different V-type ATPases. All sequences were obtained from GenBank. Regions of sequence similarity are boxed. The organism used are *Neurospora crassa* (Nc), *Saccharomyces cerevisiae* (Sc), *Manduca sexta* (Ms), and *Bos taurus* (bov).

Subunit G

Subunit G and its homologs appear to have gone through an interesting evolutionary history. This polypeptide is part of the peripheral V_1 sector of the vacuolar ATPase, yet is clearly similar in sequence to the membrane-embedded subunit b of the F-type ATPase (Lepier *et al.*, 1996; Supekova *et al.*, 1996). The major difference between these apparently homologous proteins is that the G subunit has no membrane-spanning domain. When comparing G subunits from different organisms the sequence similarity is relatively low (e.g., 31% between *N. crassa* and bovine enzymes), and the C-terminal half is poorly conserved. However, we observed that the arrangement of the conserved residues is striking. When modeled as α-helixes, the G subunits from highly divergent organisms all have one face of the helix that is essentially identical (Hunt and Bowman, 1997).

There is good biochemical evidence that the peripheral portion of the homologous b subunit forms an α-helix (Dunn, 1992). This region of the b subunit

binds to the F_1 sector and anchors it to the membrane. Subunit b is believed to form part of the stator, which prevents the α and β subunits from rotating in synchrony with the γ subunit in F-type ATPases. In the vacuolar ATPase, the G subunit could have the same function if it is bound tightly to one of the membrane-embedded polypeptides in the enzyme.

The region of sequence similarity between b and G subunits begins just after the membrane-spanning domain of the b subunits. This region must be near the membrane and is probably part of the stalk in both V- and F-type ATPases. Surprisingly, cross-linking data indicate that the site of interaction between subunit b and the α and β subunits is near the top of the F_1 sector (Ogilvie *et al.*, 1997). Although subunits b and G are large enough to extend that distance, this geometry would require that the poorly conserved region of the polypeptide form the binding site to the globular head of V_1. In the F-type ATPase from *E. coli*, the C-terminus of the b subunits has been reported to bind F_1 via the δ subunit (McLachlin *et al.*, 1998). We have no data to suggest which, if any, of the polypeptides in the vacuolar ATPase may have a similar function to the δ subunit. (Note that the homolog of the δ subunit in mitochondrial ATPases is the OSCP subunit.) Possible candidates include D, E, or the "nonhomologous region" of subunit A.

FUNCTION OF THE V_0 SUBUNITS

The membrane sector of the *N. crassa* vacuolar ATPase appears to contain at least five different types of subunits (Table I). Little is known about the arrangement of these subunits. The c subunit, a hydrophobic polypeptide that is also called the proteolipid, is clearly homologous to the subunit of the same name in F-type ATPases (Sista *et al.*, 1994). The major difference is that the vacuolar protein is twice as large and appears to have evolved from a duplication of an ancestral smaller protein. Experiments with *S. cerevisiae* have shown that two other closely related proteins, named c′ and c″, are also components of the vacuolar ATPase (Hirata *et al.*, 1997). All three share significant sequence similarity and probably evolved from a common ancestor.

Genes for all three proteins appear to be present in the *N. crassa* genome. *Vma-3* encodes subunit c. The c′ polypeptide is the product of the *vma-11* gene. We have isolated a cDNA clone and the corresponding genomic DNA. Analysis of the sequence indicates that the *N. crassa* c′ protein is more closely related to the c′ subunit of *S. cerevisiae* than it is to the *N. crassa* c subunit (J. Pounder and B. Bowman, unpublished). The c″ subunit of *S. cerevisiae* is about 20% larger than c and c′ and contains an additional membrane-spanning region (Apperson *et al.*, 1990; Hirata *et al.*, 1997). A homolog to the c″ subunit has been identified in *Magnaporthe grisea* (J. Sweigard, E.I. DuPont Nemours and Co., personal communication), a fungus closely related to *N. crassa*. We are currently using the *M. grisiea* sequence to isolate the homologous gene in *N. crassa*.

F-type ATPases contain twelve copies of the c subunit per enzyme. The bovine V-type ATPase was reported to contain 6 copies of the c subunit, consistent with the observation that the V-type c subunit is twice as large (Arai *et al.*, 1988). An interesting, unresolved question is whether the c, c′ and c″ subunits are all present in each enzyme and in what stoichiometry. In previous experiments we extracted vacuolar membranes with organic solvents, a procedure that would be expected to solubilize all three proteolipids. We digested the extract with trypsin and sequenced the fragments. All the fragments we could identify were derived from subunit c (Sista *et al.*, 1994), suggesting that subunits c′ and c″ are present in lower amounts.

The *vma-6* gene of *N. crassa* encodes a 41-kDa protein, which is tightly associated with the membrane, although it contains no obvious membrane-spanning regions (Melnik and Bowman, 1996). No homolog to this subunit has been identified in F-type ATPases. The subunit is fairly well conserved, e.g., the *N. crassa* and bovine sequences are 52% identical. However, the *N. crassa* subunit is slightly larger because of a unique glycine-rich region of 19 residues at positions 283–301. The subunit also contains three highly conserved cysteine residues. We have shown that the binding of the V_1 sector to the membrane can be affected *in vitro* by oxidizing and reducing agents (Dschida and Bowman, 1995). One speculation is that the 41-kDa protein may constitute part of the site at which V_1 binds the membrane and that its conformation can be changed *in vivo* by oxidation and reduction of the conserved cysteine residues.

The largest component of the V_0 sector is the 98-kDa subunit a encoded in *N. crassa* by the *vph-1* gene (E. J. Bowman, unpublished, GenBank accession No. U36396). As in other organisms, the N-terminal half of the polypeptide is hydrophilic, while the C-terminal half appears to contain 6–8 membrane-spanning regions. Experiments in *S. cerevisiae* have led to the

suggestion that the *vph-1* gene product has the same function as the a subunit of F-type ATPases and may form part of the proton-conducting pathway through the membrane (Leng *et al.*, 1996). Yeast has two homologs of this subunit, the products of the *VPH1* and the *STV1* genes (Manolson *et al.*, 1994). We have tried and, thus far, failed to identify an *STV1* homolog in *N. crassa*. Hybridization to genomic DNA and use of primers derived from conserved regions has produced no evidence for such a gene, nor has such a sequence appeared in data from large-scale sequencing projects.

CONCLUSIONS

Although V-type ATPases are significantly larger than F-type ATPases, the two classes of ATPases are likely to share many structural features and have the same basic mechanism. We have analyzed the vacuolar ATPase from *N. crassa* and have compared it to the V-type ATPases from other organisms. The enzyme appears to be a good general model for V-type ATPases because it has homologs to all structural subunits identified in other organisms and no known subunits unique to *N. crassa*. Six of the subunits, A, B, G, c, c′, and c″ have significant sequence similarity to subunits of the F-type ATPase and appear to be functional homologs. The remaining subunits, C, D, E, F, H, a, and d either play a unique role in the vacuolar ATPase or have significantly diverged from their counterparts in the F-type ATPase.

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