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The progenitor of ATP synthases was closely related to the current vacuolar H^+-ATPase

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The gene encoding the proteolipid of the vacuolar H^+-ATPase of yeast was cloned and sequenced. The deduced amino acid sequence of the yeast protein is highly homologous to that of the proteolipid from bovine chromaffin granules. In contrast to other membrane proteins the transmembrane segments of the bovine and yeast proteolipids were much more conserved than the hydrophilic parts. The fourth transmembrane segment, which contains the DCCD-binding site, was conserved 100%. Comparison of vacuolar and eubacterial proteolipids revealed a homology which pointed to a common ancestral gene that underwent gene duplication to form the vacuolar proteolipids. Additional support for this notion came from the amino acid sequences of subunits involved in the catalytic sectors of archaebacterial ATP synthase and plant and yeast vacuolar H^+-ATPases, which reveal extensive sequence homology. Slight, but significant, homology between the archaebacterial and eubacterial ATP synthases was observed. These observations might suggest that the progenitor of ATP synthases was closely related to the present vacuolar H^+-ATPases.

ATPase, vacuolar H^+-; ATP synthase; Proteolipid; Evolution

1. INTRODUCTION

Several of the organelles connected with the vacuolar system of eukaryotic cells contain an acidic interior [1–3]. The transmembrane proton gradient is maintained by the vacuolar H^+-ATPase through the continuous pumping of protons into the organelles [1–6]. The extent of this proton gradient is not sufficient to drive ATP formation, even at very low cellular ATP concentrations [7,8]. On the other hand, in the mitochondria, chloroplasts and bacterial cell membrane the main function of the H^+-ATPase is to form ATP at the expense of the protonmotive force generated by respiration, photosynthesis and other processes that conduct protons against the electrochemical gradient. Therefore, these proton pumps (eubacterial-type H^+-ATPases) were denoted ATP-synthases [9–12]. The activity of most of these enzymes is reversible and at low protonmotive force the enzymes pump protons while hydrolyzing ATP. The two families of proton pumps, namely the vacuolar H^+-ATPases and eubacterial H^+-ATPases, have some similar properties. Both enzymes are composed of several subunits, some of which are peripheral while others are integral membrane proteins [9–12]. The catalytic activity of these enzymes does not involve a phosphoenzyme intermediate. The proton pumping activity of both families of enzymes is inhibited by DCCD and inhibition is concomitant with covalent binding of the agent onto a specific polypeptide, the proteolipid [13–18]. Recently, cDNA encoding the proteolipid of the vacuolar H^+-ATPase from chromaffin granules was cloned and sequenced [19]. The gene encodes a highly hydrophobic 15.8 kDa polypeptide with four apparent transmembrane segments. Alignment with various proteolipids from eubacterial H^+-ATPases

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Abbreviations: F-ATPase, the eubacterial type H^+-ATPase (F_0F_1); V-ATPase, vacuolar type H^+-ATPase; DCCD, *N,N'*-dicyclohexylcarbodiimide

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revealed that all of them may evolve from a common ancestral gene that underwent gene duplication to form the vacuolar proteolipids. In this work we report on cloning and sequencing of the yeast vacuolar proteolipid. The evolutionary relations among the vacuolar and eubacterial proton pumps is discussed.

2. MATERIALS AND METHODS

2.1. *Materials*

Most of the chemicals were purchased from Sigma. Most of the enzymes were purchased from BRL and New England Biolabs. The radioactive materials were from Amersham. Nitrocellulose membranes were used for the dot blot and Southern analysis and Colony Screen membranes from NEN Research products were used to screen the library.

2.2. *Analytical methods*

Published procedures were used for end labeling of DNA probes and nick-translation [20]. Screening of the library, dot blot analysis, Southern hybridization and cloning into M13 were performed according to Maniatis et al. [20].

2.3. *Cloning the gene encoding the proteolipid of the vacuolar H^+-ATPase in yeast*

A DNA probe was synthesized according to 35 amino acids of the C-terminus of the proteolipid from bovine chromaffin granules [19], and the most frequent codon usage in yeast. The probe had the following sequence: ACTGCTCAACAACCAAGATTATTCGTTGGTATGATTTTAATTTTAATTTTGGCTGAAGTTTTAGGTTTATACGGTTTAATTGTTGCTTTAATTTTATCTACTAAA. A yeast (*Saccharomyces cerevisiae*) genomic library, kindly donated by Dr Mark Rose of Princeton University, was screened with ^{32}P end labeled oligonucleotides. The library was constructed by cloning *Sau*3A fragments of yeast genomic DNA into the *Bam*HI site of YCp50 and the inserts to be cut out by *Eco*RI and *Sph*I [21]. The positives were confirmed by dot blots and Southern hybridization. Two of them with identical restriction sites contained a 1.4 kb *Eco*RI-*Eco*RI DNA segment, which was isolated and cloned into M13. The DNA fragment was sequenced by the dideoxy chain-termination technique following the generation of overlapping deletions by digestion with exonuclease III [22,23]. About 0.4 kb of the insert was part of the tetracycline resistance gene from the plasmid.

3. RESULTS

Genomic DNA library of yeast cells (*S. cerevisiae*) was screened by ^{32}P end labeled DNA probe. Several positives were detected and checked by dot blot and Southern hybridization with the same probe. Two of the plasmids, isolated from the positives, had the same restriction sites pattern and on the Southern hybridization a 1.4 kb *Eco*RI-*Eco*RI fragment lit up. This fragment was isolated, subcloned into M-13 and sequenced. Fig.1 shows the nucleotide and the deduced amino acid sequences of the gene. The DNA sequence contains an open reading frame of 160 amino acids, which correspond to a polypeptide of 16352 Da. Hydrophobicity plots of the proteolipids from yeast and bovine chromaffin granules are depicted in fig.2. A remarkable similarity between the two patterns is observed, and like the proteolipid from

```
TTTTTCCCGGAAAAGGAAACGGCATTTAAAAAAAAAAAAAAAAAAAAAAACAGAAAAAGTGGTTAAGTGATTGACTGACCCTTGATAGTTTTGTACAATTA  100

TACACTCGTTCTGATTAAAAACTTGTTTATAAAAATCTTTTTAAAAGAAAGAGAAGATCGTGTTTATTGCTTTTCTCAAAAAGACTAATCAATTAGAATA  200

ACAAAAGAAACATATACATATAATGACTGAATTGTGTCCTGTCTACGCCCCTTTCTTTGGTGCCATTGGTTGTGCCTCTGCAATTATCTTCACCTCATTA  300
                      M  T  E  L  C  P  V  Y  A  P  F  F  G  A  I  G  C  A  S  A  I  I  F  T  S  L

GGTGCTGCTTACGGTACTGCTAAGTCTGGTGTTGGTATCTGTGCCACTTGTGTGTTGAGACCAGACCTATTATTCAAGAACATTGTTCCTGTTATTATGG  400
G  A  A  Y  G  T  A  K  S  G  V  G  I  C  A  T  C  V  L  R  P  D  L  L  F  K  N  I  V  P  V  I  M  A

CTGGTATCATTGCCATTTACGGTTTAGTTGTTTCCGTTTTGGTTTGTTATTCGTTGGGTCAAAAGCAAGCTTTGTACACCGGTTTCATCCAATTGGGTGC  500
  G  I  I  A  I  Y  G  L  V  V  S  V  L  V  C  Y  S  L  G  Q  K  Q  A  L  Y  T  G  F  I  Q  L  G  A

CGGTCTATCAGTCGGTTTGAGTGGTCTAGCTGCTGGTTTCGCTATTGGTATTGTCGGTGATGCAGGTGTTAGAGGTTCCTCTCAACAACCAAGATTATTC  600
 G  L  S  V  G  L  S  G  L  A  A  G  F  A  I  G  I  V  G  D  A  G  V  R  G  S  S  Q  Q  P  R  L  F

GTCGGTATGATTTTGATTTTGATTTTTGCTGAAGTTTTGGGTCTATACGGTTTGATTGTTGCTTTGTTGTTGAACTCCAGGGCTACTCAAGATGTTGTCT  700
V  G  M  I  L  I  L  I  F  A  E  V  L  G  L  Y  G  L  I  V  A  L  L  L  N  S  R  A  T  Q  D  V  V  C

GTTAAGGCAGCTTCTGAATCACTAAAGCAGGAATAGAGTATACAAAAGAATCTTTTTGTGAGAAACTATGAAAACTATCAAAATTTTTCATCTTAGAGAT  800

TGTTTATAGCCCCTATTCTTACTTCATATCCAAACTACCTCATTTCAAGCTTCCTTTTTATTAGTACCATTATTATTGTTTAAAAGAGTATTACACGCTA  900

AATATGTATATCTTAATTTTTAATGTGTATAAAATAGAAAATTTGAATATCAAGGAGCAACAAAGAGCATTATGCGTTTTAAGCTACACAATGGGTACGA  1000

TGAGATC
```

Fig.1. The DNA and amino acid sequences of the gene encoding the proteolipid subunit of the yeast vacuolar H^+-ATPase.

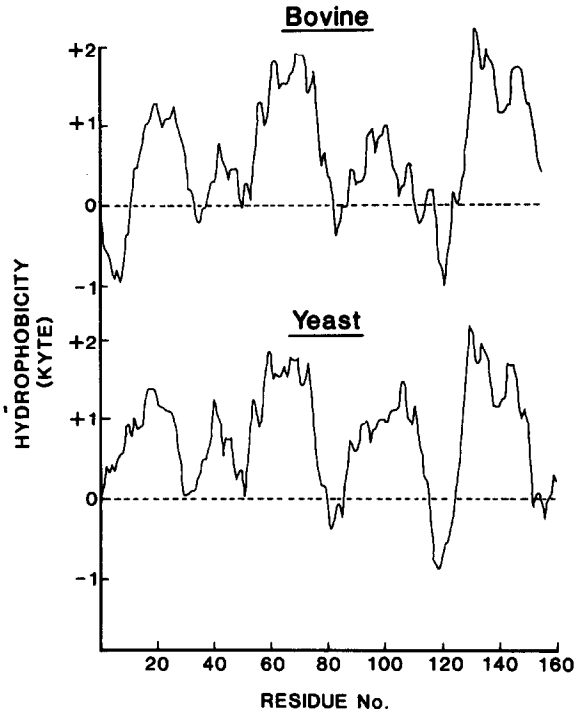

Fig.2. Hydropathic profile of the yeast proteolipid in comparison with the proteolipid of the bovine chromaffin granules H^+-ATPase. Analysis was performed by a DNASTAR program according to Kyte and Doolittle [44], using window size of 11 amino acids.

chromaffin granules, the yeast proteolipid shows four distinct transmembrane segments [19]. Alignment of the two amino acid sequences is shown in fig.3. Four highly homologous stretches of sequences corresponding to parts of the transmembrane segment are present. The fourth segment which contains the DCCD-binding site [19], is 100% conserved. Thus, vacuolar H^+-ATPases show remarkable amino acid conservation in the proteolipid subunits of the enzyme, and in contrast to globular proteins the hydrophobic parts are more conserved than the hydrophilic ones. Southern blots of yeast DNA digested with four different restriction enzymes, probed by ^{32}P-labeled DNA encoding the proteolipid, revealed only a single copy of this gene in the yeast genome (fig.4). This implies that an identical membranal moiety of the enzyme is pumping protons into the various organelles of the vacuolar system.

Recent studies demonstrated that in analogy to the eubacterial H^+-ATPases, the vacuolar enzymes can be separated into two distinct sectors [24–27]: a membrane sector composed of the proteolipid and probably an additional hydrophobic polypeptide of 20 kDa, and a catalytic sector composed of five different polypeptides [27]. It was shown that among these subunits polypeptides of about 72 kDa (A) and 57 kDa (B) contain the active site of the vacuolar enzymes [5,30,31]. The comparison of the 57 kDa subunit of the recently sequenced yeast vacuolar H^+-ATPase [28] with a sequence of the same subunit from *Arabidopsis* [29], leads us to believe that the whole enzyme was conserved. Moreover, the conservation is not limited only to the vacuolar H^+-ATPase from

```
                        10        20        30        40        50        60        70        80
                        v         v         v         v         v         v         v         v
Yeast H+-ATPase   MTELCPVYAPFFGAIGCASAIIFTSLGAAYGTAKSGVGICATCVLRPDLLFKNIVPVIMAGIIAIYGLVVSVLVCYSLGQ
                    :  * **:**: :* ::*::*::********** ** *  *:**::: *:*:**:***********:**:  ** :
Bovine H+-ATPase  MSEAKNGPEYASFFAVMGASAAMVFSALGAAYGTAKSGTGIAAMSVMRPEMIMKSIIPVVMAGIIAIYGLVVAVLIANSLND
                        ^         ^         ^         ^         ^         ^         ^         ^
                        10        20        30        40        50        60        70        80

                        90        100       110       120       130       140       150       160
                        v         v         v         v         v         v         v         v
KQALYTGFIQLGAGLSVGLSGLAAGFAIGIVGDAGVRGSSQQPRLFVGMILILIFAEVLGLYGLIVALLLNSRATQDVVC
  :** :*:*************** : :::* * ::*****************************:*:::
GISLYRSFLQLGAGLSVGLSGLAARSPSALLGTQGRACTAQQPRLFVGMILILIFAEVLGLYGLIVALILSTK
        ^         ^         ^         ^         ^         ^         ^
        90        100       110       120       130       140       150
```

Fig.3. Alignment of the amino acid sequences of the proteolipids from yeast and bovine chromaffin granules. The sequences were aligned using the Align program of DNASTAR. Identical amino acids are indicated by (*) and conservative amino acid replacements are indicated by (:).

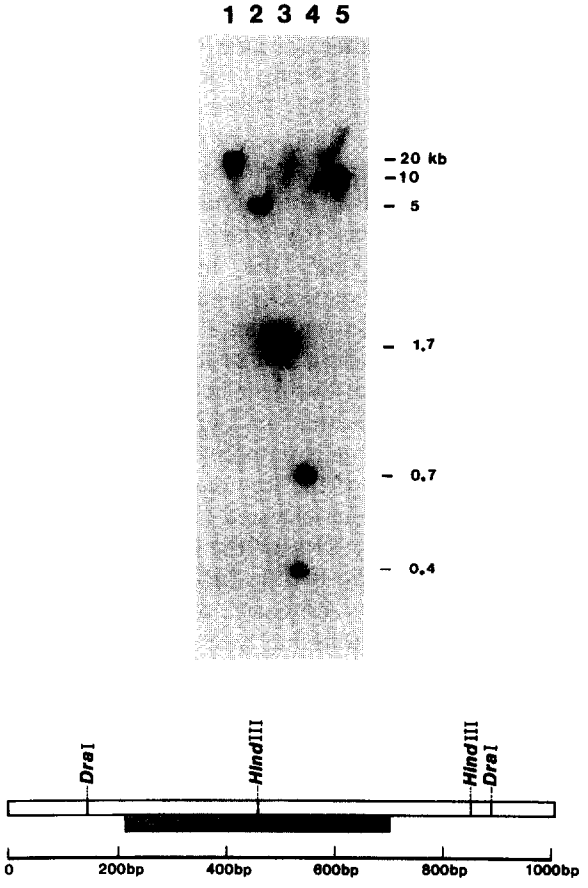

Fig.4. Southern blot of genomic DNA from *S. cerevisiae* probed by the gene encoding the proteolipid. Genomic DNA was digested with *Bam*HI (lane 1), *Bgl*II (lane 2), *Eco*RI (lane 3), *Hin*dIII (lane 4) and *Pst*I (lane 5). The digested DNA was electrophoresed on a 0.8% agarose gel, transferred to nitrocellulose and probed with a 0.74 kb *Dra*I fragment containing the proteolipid gene, which had been labeled with ^{32}P by nick-translation. At the bottom the position of the restriction sites of *Dra*I and *Hin*dIII are indicated.

various sources, but exists also between the ATP syntheses and the vacuolar H^+-ATPases. Subunit A (72 kDa) of the vacuolar enzymes contains both the nucleotide binding site and a reactive -SH group, upon which binding *N*-ethylmaleimide results in inhibition of the enzyme [5,30,31]. The gene from carrots encoding this polypeptide was recently cloned and sequenced [32]. A weak but significant homology with the β-subunit of the eubacterial enzymes was detected. Similarly, the gene encoding a subunit of the ATP-synthase of the archaebacterium *Sulfolobus acidocaldaris* was found to exhibit some homology with β-subunits of chloroplast, mitochondria and eubacterial H^+-ATPases [33]. However, alignment of the amino acid sequences of the carrot and *Sulfolobus* polypeptides revealed about 50% sequence identity along the entire length of the polypeptides (not shown). Amino acid alignment of part of subunit A of the H^+-ATPase from carrot and *Sulfolobus* with sequences of the β-subunit of the *E. coli* enzyme showed significant homology [32,33]. The consensus amino acid sequence for the ATP-binding site was conserved in all of those polypeptides.

Subunit B (57 kDa) was shown to be a translation product of a very conserved gene [28,29,34]. Recently the gene encoding subunit B of the ATP synthase of *Sulfolobus* was cloned and sequenced [35]. As shown in fig.5, the deduced amino acid sequence is highly homologous to the amino acid sequence of subunit B of the vacuolar H^+-ATPase from yeast. Moreover, these polypeptides contain regions of homology with the α- and β-subunit of H^+-ATPases from mitochondria, chloroplasts and bacteria [28]. The regions of α- and β-subunits of F-ATPases that showed the highest homology with subunit B of the vacuolar enzyme showed significant homology among themselves. These results strongly suggest that the ATP-synthases are related to the present vacuolar H^+-ATPases of eukaryotic cells, and that the α- and β-subunits of F-ATPases as well as the A and B subunits of V-ATPases evolved from a common ancestral protein that was closely related to subunit B of the current vacuolar enzymes.

4. DISCUSSION

Recently, the cDNA encoding the proteolipid of the vacuolar H^+-ATPase from bovine chromaffin granules was cloned and sequenced [19]. The gene encodes a highly hydrophobic protein with a molecular mass of 15849 Da, which is double the size of the proteolipids from eubacterial type enzymes. Moreover, hydropathy plots revealed four transmembrane segments, which are twice the number of transmembrane segments of the proteolipids from mitochondria, chloroplasts and bacteria [19,36,37]. It was concluded that the proteolipids of the eubacterial and vacuolar

Fig.5. Alignment of the amino acid sequences of subunit B of the H^+-ATPases from *Saccharomyces cerevisiae* and *Sulfolobus* and the α- and β-subunits of the enzyme of *E. coli*. The sequences were taken from [10,28,35]. Amino acid sequences of the entire B subunit of *Sulfolobus* and yeast are presented and sequences of the indicated stretches of the α- and β-subunits of *E. coli* BF_1 are aligned to them.

them.

H^+-ATPases evolved from a common ancestral gene that existed prior to the segregation of various organelles [19]. The data presented in this paper gives credence to this suggestion. Extrapolating the high degree of conservation among the vacuolar H^+-ATPases, together with the high homology among them in comparison with the archaebacterial ATP-synthase, suggests that the progenitor of these proton pumps was closely related to the current vacuolar enzymes.

A putative rooted tree, adopted from Zillig et al. [38], which represents the evolution of the eubacterial and vacuolar H^+-ATPases, is depicted in fig.6. We propose that the proton-ATPase of the progenote was similar to the present vacuolar H^+-ATPases. The function of that enzyme was to balance the interior pH of the organism and to produce ATP by harvesting the protonmotive force generated by electron transport across their membrane. Step 1 represents the evolutionary pathway of the H^+-ATPase of the progenote into the enzyme present in the current archaebacteria. The subunits of the archaebacterial enzyme were somewhat shortened and evolved through small changes especially in their terminal sequences. The enzyme functions in both balancing the interior pH and ATP synthesis. Step 2 shows the evolutionary pathway of the vacuolar H^+-ATPases in eukaryotic cells. Very small changes in the subunit composition and amino acid sequences took place. The progenote's ATP synthase evolved into the vacuolar H^+-ATPase of eukaryotes by duplicating the gene encoding the proteolipid [19]. We suggest that this renders the vacuolar enzyme into a proton pump that can no longer synthesize ATP. In addition to the gene duplication structural changes led to the development of strict control over the extent of acidification through the introduction of a regulated slip which dissipates excessive proton gradients [7,8]. Step 3 represents the evolution of the basic eubacterial ATP synthase (H^+-ATPase). The fundamental structure of the catalytic sector composed of five different polypeptides (α to ϵ), and the membrane sector of three different polypeptides (a to c), is formed. Major changes in the size and amino acid sequences took place. The α- and β-subunits of the F-ATPase evolved from the progenote's 57 kDa polypeptide that underwent gene duplication to give two non-identical subunits. The β-subunit of the eubacterial enzyme

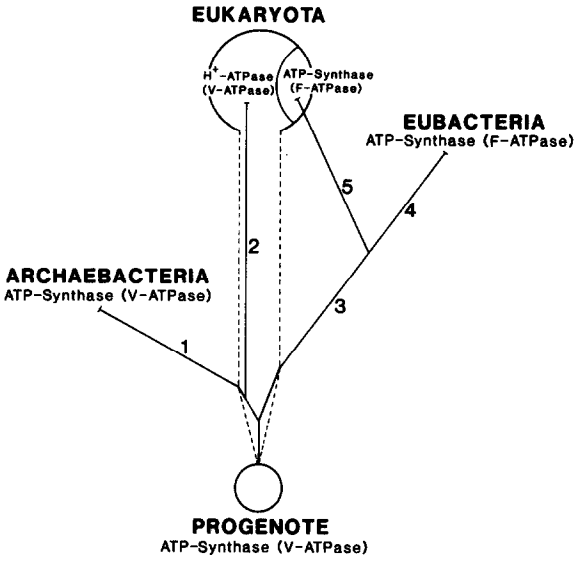

Fig.6. A proposed rooted evolutionary tree of the two families of proton pumps. V-ATPase denotes the enzymes which are structurally related to the present vacuolar H^+-ATPases. F-ATPase denotes the enzymes which are structurally related to the present eubacterial H^+-ATPase. ATP synthase indicates that the enzyme can function in ATP formation at the expense of the protonmotive force. The rooted tree was adopted from Zillig et al. [38]. The explanation for the roots that are marked by the numbers 1 to 5 is given in the text.

evolved by deletion of amino acids especially in the middle of the polypeptide. Perhaps the changes led to better coupling between the proton conduction and ATP formation. Step 4 depicts the evolution of the current ATP synthase of the bacterial cell membrane, that functions in proton pumping and/or ATP formation. Small changes took place for adapting the enzyme to the specific growing conditions of the various bacteria. Step 5 represents the evolution of the eubacterial enzyme into the ATP synthase of mitochondria and chloroplasts. Photosynthetic bacteria established endosymbiosis with primordial eukaryotic cells to form the semiautonomous organelles [39,40]. Some of the genes encoding subunits of the bacterial ATP synthase were transferred to the nucleus of the eukaryotic cell. Gene duplication of the b-subunit and transformation of one of the copies to the nucleus form the present H^+-ATPase of chloroplasts [41,42]. Major rearrangement of the subunits and addition of several new polypeptides formed the mitochondrial enzyme. As with

other membrane protein complexes, advancement in evolution adds more subunits serving for better regulation of the system and more efficient biogenesis and assembly of the protein complex [43].

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REFERENCES

[1] Al-Awqati, Q. (1986) Annu. Rev. Cell Biol. 2, 179–199.
[2] Mellman, I., Fuchs, R. and Helenius, A. (1986) Annu. Rev. Biochem. 55, 663–700.
[3] Rudnick, G.A. (1986) Annu. Rev. Physiol. 48, 403–413.
[4] Njus, D., Kelley, P.M. and Harnadek, G.J. (1986) Biochim. Biophys. Acta 853, 237–265.
[5] Bowman, B.J. and Bowman, E.J. (1986) J. Membr. Biol. 94, 83–97.
[6] Nelson, N. (1988) Plant Physiol. 86, 1–3.
[7] Moriyama, Y. and Nelson, N. (1987) J. Biol. Chem. 262, 9175–9180.
[8] Moriyama, Y. and Nelson, N. (1988) in: The Ion Pumps, Structure, Function and Regulation (Stein, W.D. ed.) pp.377–409, Alan R. Liss, Inc., New York.
[9] Racker, E. (1976) A New Look at Mechanism in Bioenergetics, Academic Press, New York.
[10] Futai, M. and Kanazawa, H. (1983) Microbiol. Rev. 47, 285–312.
[11] Nelson, N. and Cidon, S. (1984) J. Bioenerg. Biomembr. 16, 11–36.
[12] Walker, J.E., Saraste, M. and Nicholas, J.F. (1984) Biochim. Biophys. Acta 768, 164–200.
[13] Bowman, E.J. (1983) J. Biol. Chem. 258, 15238–15244.
[14] Uchida, E., Ohsumi, Y. and Anraku, Y. (1985) J. Biol. Chem. 260, 1090–1095.
[15] Manolson, M.F., Rea, P.A. and Poole, R.J. (1985) J. Biol. Chem. 260, 12273–12279.
[16] Randall, S.K. and Sze, H. (1986) J. Biol. Chem. 261, 1364–1371.
[17] Arai, H., Berne, M. and Forgac, M. (1987) J. Biol. Chem. 262, 11006–11011.
[18] Sun, X.-Z., Xie, X.-S. and Stone, D.K. (1987) J. Biol. Chem. 262, 14790–14794.
[19] Mandel, M., Moriyama, Y., Hulmes, J.D., Pan, Y.-C.E., Nelson, H. and Nelson, N. (1988) Proc. Natl. Acad. Sci. USA 85, 5521–5524.
[20] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, New York.
[21] Rose, M.D. and Fink, G.R. (1987) Cell 48, 1047–1060.
[22] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[23] Henikoff, S. (1984) Gene 28, 351–359.
[24] Rea, P.A., Griffith, C.J., Manolson, M.F. and Sanders, D. (1987) Biochim. Biophys. Acta 904, 1–12.
[25] Arai, H., Terres, G., Pink, S. and Forgac, M. (1988) J. Biol. Chem. 263, 8796–8802.
[26] Xie, X.-S. and Stone, D.K. (1988) J. Biol. Chem. 263, 9859–9867.
[27] Moriyama, Y. and Nelson, N. (1989) J. Biol. Chem., in press.
[28] Nelson, H., Mandiyan, S. and Nelson, N. (1989) J. Biol. Chem. 264, 1775–1778.
[29] Manolson, M.F., Quellette, B.F.F., Filion, M. and Poole, R.J. (1988) J. Biol. Chem. 263, 17989–17994.
[30] Uchida, E., Ohsumi, Y. and Anraku, Y. (1988) J. Biol. Chem. 263, 45–51.
[31] Moriyama, Y. and Nelson, N. (1987) J. Biol. Chem. 262, 14273–14279.
[32] Zimniak, L., Dittrich, P., Gogarten, J.P., Kibak, H. and Taiz, L. (1988) J. Biol. Chem. 263, 9102–9112.
[33] Denda, K., Konishi, J., Oshima, T., Date, T. and Yoshida, M. (1988) J. Biol. Chem. 263, 6012–6015.
[34] Bowman, B.J., Allen, R., Wechser, M.A. and Bowman, E.J. (1988) J. Biol. Chem. 263, 14002–14007.
[35] Denda, K., Konishi, J., Oshima, T., Date, T. and Yoshida, M. (1988) J. Biol. Chem. 263, 17251–17254.
[36] Hoppe, J. and Sebald, W. (1984) Biochim. Biophys. Acta 768, 1–27.
[37] Schneider, E. and Altendorf, K. (1987) Microbiol. Rev. 51, 477–497.
[38] Zillig, W., Palam, P., Deiter, W.-D., Gropp, F., Puhler, G. and Klenk, H.-P. (1988) Eur. J. Biochem. 173, 473–482.
[39] Margulis, L. (1974) Evol. Biol. 7, 45–78.
[40] Raven, P.H. (1970) Science 169, 641–646.
[41] Cozens, A.L., Walker, J.E., Phillips, A.L., Huttly, A.K. and Gray, J.C. (1986) EMBO J. 5, 217–222.
[42] Berzborn, R.J., Otto, J., Finke, W., Meyer, H.E. and Block, J. (1987) Biol. Chem. Hoppe Seyler 368, 551–552.
[43] Nelson, N. and Cidon, S. (1984) J. Bioenerg. Biomembr. 16, 11–36.
[44] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.