Planta (1990) 180:361–371

Planta

Occurrence of the 32-kDa Q_B-binding protein of photosystem II in vegetative cells, heterocysts and akinetes of *Azolla caroliniana* cyanobionts

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Abstract. Transmission electron microscopy and immunocytological labeling were used to localize the 32-kilodalton (kDa) protein (DI polypeptide) of photosystem II in different cell types of the cyanobionts within leaf cavities of *Azolla caroliniana* Willd. The 32-kDa protein binds the secondary electron acceptor Q_B, and is highly conserved between plants and cyanobacteria. Three antisera, specific for different epitopes of the 32-kDa protein, were used as primary antibodies. Immunologically recognizable 32-kDa protein was localized on membranes of *Azolla* chloroplasts, vegetative cyanobacterial cells, akinetes, and heterocysts that were at all stages of the differentiation process. The 32-kDa protein was not detected in nonphotosynthetic endosymbiotic bacteria found within leaf cavities. The amount of the 32-kDa protein observed in different cyanobacterial cell types was dependent upon the primary antiserum used and membrane orientation within a cell with respect to the plane of sectioning. Therefore, although 32-kDa protein was present in all three cyanobacterial cell types and clear trends in labeling patterns could be elucidated, it was not possible to quantitate the amounts of protein with respect to either cell type or leaf-cavity age.

Key words: *Anabaena* – *Azolla* – Cyanobacteria – Heterocyst – Protein, 32-kDa – Symbiosis (*Azolla-Anabaena*)

Introduction

The genus *Azolla* comprises a group of small, aquatic ferns having multibranched, floating stems bearing alternate pairs of deeply bilobed leaves. Each leaf consists of a dorsal and a ventral lobe. The thin, transparent ventral leaf lobes float on the surface of water, while the dorsal lobes are thicker, aerial, and enclose extracellular cavities containing hair cells, nitrogen-fixing cyanobacterial endosymbionts, and several types of bacteria.

Azolla and its cyanobacterial endosymbionts exhibit synchronous development (Hill 1975, 1977; Becking 1987). Small, undifferentiated cyanobacterial cells, which are ultrastructurally similar, are found adjacent to the apical meristem and each *Azolla* shoot tip. In contrast, mature leaf cavities commonly contain young, undifferentiated vegetative cells, older stationary-phase vegetative cells, heterocysts (at various stages of differentiation) and, in some *Azolla* spp., developing or mature akinetes (see Braun-Howland and Nierzwicki-Bauer 1990).

Heterocysts are terminally differentiated cells which, in most free-living (Wolk 1982; Murry et al. 1984; Golden et al. 1985) and symbiotic (Bergman et al. 1986; Braun-Howland et al. 1988) heterocystous cyanobacteria, are the exclusive sites of nitrogen fixation (however, see Rippka and Stanier 1978). In order to protect the oxygen-sensitive enzyme nitrogenase, heterocyst differentiation is accompanied by substantial subcellular and metabolic reorganization (Alberte et al. 1980; Haselkorn et al. 1984; see Adams and Carr 1981). Of particular interest is the apparent loss of O_2-evolving activity associated with Photosystem II (PSII) (Bradley and Carr 1971; Donze et al. 1972; Tel-Or and Stewart 1977; Almon and Böhme 1980; Peterson et al. 1981).

The 32-kDa protein is a core protein of PSII which binds the secondary electron acceptor Q_B (Arntzen et al. 1982) and is the site of action for several herbicides (see Allen 1983). The results of molecular studies (Nierzwicki-Bauer and Haselkorn 1986) indicated that *psb*A gene transcripts, encoding the 32-kDa protein of PSII, were seven to ten times more abundant in *A. caroliniana* symbionts than in free-living *Anabaena azollae* isolated from *A. caroliniana*. This result was unexpected because heterocysts comprise up to 30% of the total cell population in *Azolla* cyanobionts, versus approx. 10% in free-living *Anabaena azollae* grown under nitrogen-limiting

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Abbreviations: Ab = antibody; kDa = kilodalton(s); PSII = photosystem II

conditions. To explain these results, it was postulated that transcription of the *psb*A genes might also occur in heterocysts of symbiotic *Anabaena*, where the 32-kDa protein would have a function different from that which it has in vegetative cells (Nierzwicki-Bauer and Haselkorn 1986).

Akinetes (cyanobacterial spores) are specialized cells produced by certain genera of filamentous, heterocystous cyanobacteria as a means of withstanding exposure to desiccation or low temperatures (Yamamoto 1975; Sutherland et al. 1979). They are ultrastructurally similar to stationary-phase vegetative cells, except that most akinetes are surrounded by a thick cell-wall layer and extracellular envelope. The precise factors governing akinete differentiation have not been elucidated, but light limitation is considered to be an important factor in some cyanobacterial species (see Herdman 1987). In *A. caroliniana* cyanobionts, akinetes are first observed at approx. leaf 4 from the apical meristem (Nierzwicki-Bauer et al. 1989), and have been estimated to comprise approx. 17% of the cyanobacterial cell population (see Peters 1975).

The primary objective of the present study was to determine the distribution of the 32-kDa protein in vegetative cells, heterocysts and akinetes of the *A. caroliniana* cyanobionts. A secondary objective was to ascertain whether the increased *psb*A transcript levels observed in symbiotic versus free-living *Anabaena azollae* were reflected by large quantities of 32-kDa protein in heterocysts of the cyanobionts. To achieve these objectives, antisera directed against the 32-kDa Q_B-binding protein of PSII were used in immunocytological labeling studies to localize expression of *psb*A gene transcripts within different types of cyanobacterial cells.

Material and methods

Organisms and growth conditions

Azolla caroliniana Willd. (International Rice Research Institute (IRRI), Los Baños, The Philippines; collection No. 3001), originally collected in Virginia, USA (Peters and Mayne 1974), was used in this study. Plants were grown in 2.8-l Fernbach flasks containing 1 l of IRRI medium lacking combined nitrogen (Watanabe et al. 1977) in a controlled-environment room under conditions described in Braun-Howland et al. (1988).

Fixation, embedding and immunocytological labeling

Actively growing *A. caroliniana* plants were fixed in the dark using 2.5% glutaraldehyde in 75 mM Na-cacodylate, pH 7.4, for 1 h. Infiltration and embedding of *A. caroliniana* in Araldite-Embed 812 (EPON) (Electron Microscopy Sciences, Fort Washington, Penn., USA) were performed after dehydration of samples in a graded ethanol series followed by propylene oxide. Thin sections were cut with a diamond knife and retrieved on 200-mesh (approx. 80 lines $\cdot m^{-1}$) nickel grids (Electron Microscopy Sciences) which had been coated with gold-palladium (Ernest Fullam, Latham, N.Y., USA) using a high-vacuum evaporator (Model VE 300; Veeco Instruments, Long Island, N.Y., USA). Samples were viewed and photographed using a JEM-100 CXII transmission electron microscope (JEOL USA, Peabody, Mass., USA) operating at an accelerating potential of 80 kV.

Immunocytological labeling, including controls, was performed as described previously for the cyanobionts of *A. caroliniana* and *A. pinnata* (Braun-Howland et al. 1988) using the primary antibodies described below. Secondary antibodies, goat anti-rabbit immunoglobulin G (IgG) conjugated to 15-nm colloidal gold particles (GAR 15; Janssen Pharmaceutica, Beerse, Belgium) were employed at a dilution of 1:20 for detection of dinitrogenase reductase and the 32-kDa protein when either Antibody 1 (Ab 1) or Ab 2 (see below) were used as the primary antibody. When Ab 3 was used as the primary antibody, secondary antibodies were employed at a dilution of 1:15. In controls, performed by omission of primary antisera, no labeling was observed. After immunolabeling, sections were poststained for 15 min with 4% aqueous uranyl acetate.

Primary antibodies

Dinitrogenase reductase. Antibodies (1:100 dilution) directed against the dinitrogenase reductase component (Fe-protein) of nitrogenase purified from *Azotobacter vinelandii* were kindly donated by Dr. J. Oelze, Institut für Biologie II, Universität Freiburg, FRG. This antibody preparation has been characterized previously using immunoblotting (Bühler et al. 1987).

32-kDa Q_B-binding protein. Three antibody preparations, directed against different portions of the 32-kDa protein, were used as primary antisera. Antibody preparation No. 1, designated Ab 1, was raised in rabbits against a synthetically produced peptide found within the hydrophilic, carboxyl-terminal end (amino acids 333–342) of the protein (Mei et al. 1989). It was employed at a dilution of 1:30000. Antibody preparation No. 2 (Ab 2) was raised against a site-specific, synthetic peptide (amino acids 127–136) found within a hydrophilic loop (Qb-4) connecting transmembrane spans of the 32-kDa protein (Sayre et al. 1986). It was employed at a dilution of 1:35000. Antibody 1 and Ab 2 were kindly donated by Dr. R. Sayre (Departments of Botany and Biochemistry, Ohio State University, Columbus, USA). The third antibody preparation used in these studies, Ab 3, was raised against the carboxyl-terminal two-thirds of the 32-kDa protein from *Amaranthus hybridus* (Ohad et al. 1985) and generously donated by Dr. L. McIntosh, MSU-DOE Plant Research Laboratories, Michigan State University, East Lansing, USA. It was used at a concentration of 200 µg lyophilized antiserum $\cdot ml^{-1}$.

Results

Like other PSII core proteins, the 32-kDa protein shows a high degree of phylogenetic conservation (Hoffman-Falk et al. 1982). In previous studies (Ohad et al. 1985; Jansson et al. 1987; Goloubinoff et al. 1988) it has been demonstrated that Ab 3 (Ohad et al. 1985) cross-reacts specifically with cyanobacterial *psb*A gene products. Using immunoblotting techniques, we additionally confirmed that antibodies raised against synthetically produced 32-kDa-specific peptides (Sayre et al. 1986; Mei et al. 1989) cross-react monospecifically with 32-kDa protein of the *A. caroliniana* cyanobionts (data not shown).

Transmission electron microscopy (TEM) results of immunolabeling studies in a young *Azolla* leaf cavity using an antiserum specific for a peptide within the hydrophilic carboxyl-terminal portion of the 32-kDa pro-

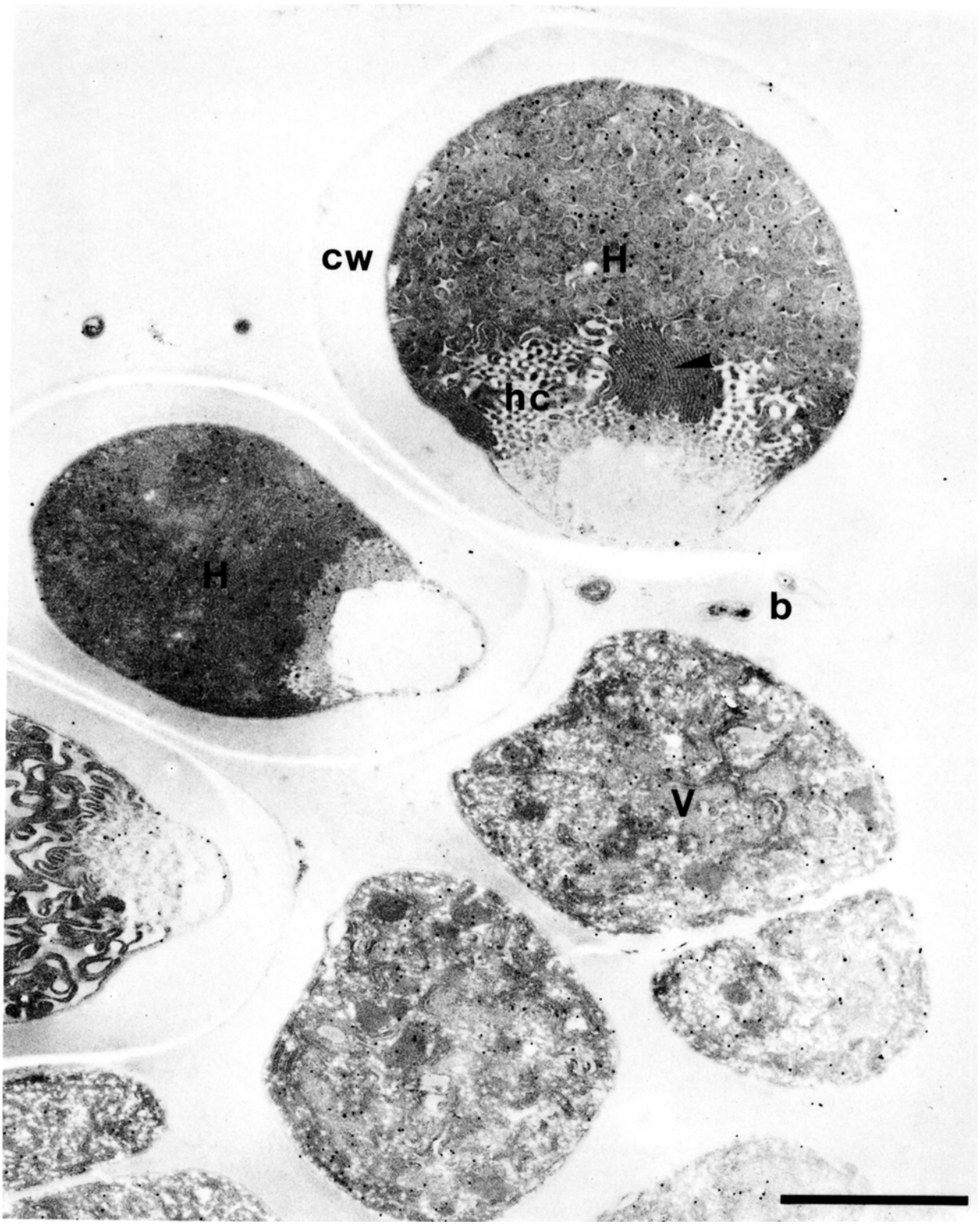

Fig. 1. Immunogold labeling of the 32-kDa protein of PSII in *A. caroliniana* endosymbionts. An oblique section through vegetative cells, heterocysts, and bacteria in which Ab 1, specific for a peptide within the hydrophilic carboxyl-terminal end of the 32-kDa protein, has been used as the primary antibody. Gold particles are localized on membranes within both vegetative cells (*V*) and mature heterocysts (*H*). Heterocysts are clearly distinguishable by the presence of extra cell-wall layers (*cw*) and stacked membranes (*arrowhead*) in the polar regions which, when observed in cross-section, appear as honeycomb configurations (*hc*). Bacteria (*b*) present within the *Azolla* leaf cavity do not label above biological background levels. Bar = 1 μm; × 31600

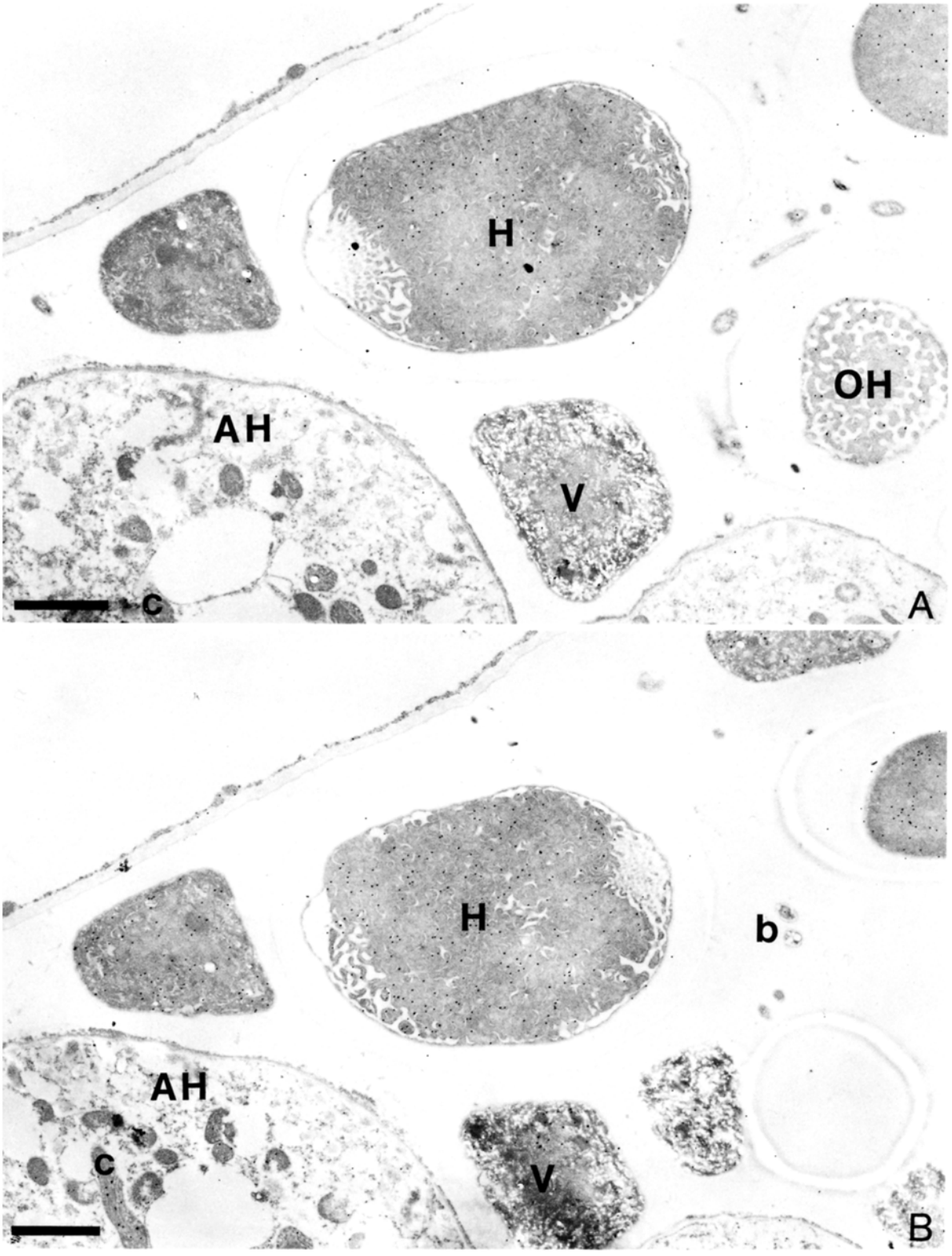

Fig. 2A, B. Immunogold labeling of serial sections through an *Azolla* leaf cavity using antibodies directed against either the dinitrogenase reductase (Fe-protein) component of nitrogenase or the 32-kDa protein of PSII using Ab 1. Bars = 1 μm. **A** The presence of dinitrogenase reductase was restricted to mature (*H*) and old (*OH*) heterocysts of the *Azolla* cyanobionts. Immunogold label was absent from vegetative cells (*V*) and *Azolla* hair cells (*AH*) containing chloroplasts (*c*). ×15900. **B** Localization of 32-kDa protein in a serial section through the same group of cells shown in **A**. Heterocysts (*H*) which contain the Fe-protein of nitrogenase (see Fig. 3A) also contain the 32-kDa protein. In addition, the 32-kDa protein was detected in vegetative cells (*V*) and within chloroplasts (*c*) of *Azolla* hair cells (*AH*), but not above background levels in bacteria (*b*). ×14500

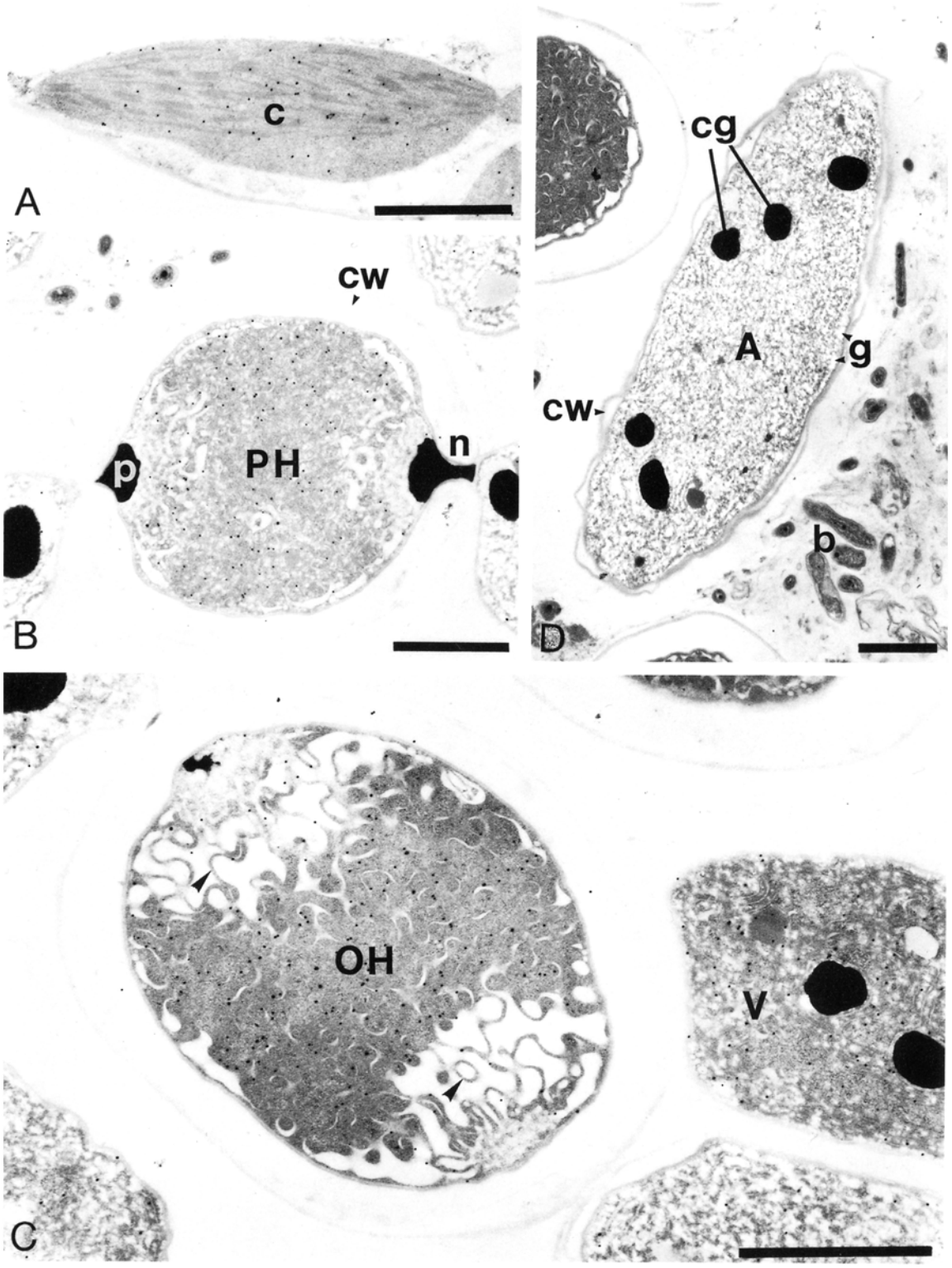

Fig. 3A–D. Localization of 32-kDa protein in different cyanobacterial cell types and in *Azolla* chloroplasts using Ab 2, directed against a hydrophilic epitope contained within a transmembrane loop of the 32-kDa protein. Bars = 1 μm. **A** Longitudinal section through an *Azolla* chloroplast (*c*). Label is observed on both granal and stromal membranes. × 22300. **B** Immunogold labeling of 32-kDa protein in a longitudinal section through a proheterocyst (*PH*) of the *A. caroliniana* cyanobiont. Thick extra cell-wall layers (*cw*), narrowed necks (*n*) and polar plugs (*p*) are ultrastructural features of proheterocysts. × 18600. **C** The 32-kDa protein was also detected within younger vegetative cells (*V*) and old heterocysts (*OH*). Extensive membrane vesiculation beginning at the cell poles (*arrowheads*) is an ultrastructural characteristic of older heterocysts. × 31000. **D** Immunogold labeling of a developing akinete (*A*). When labeled with Ab 2, very little 32-kDa protein was detected within akinetes, which possess extra cell-wall layers (cw), numerous glycogen granules (*g*) and cyanophycin granules (*cg*). Bacteria (*b*) do not label above background levels. × 12400

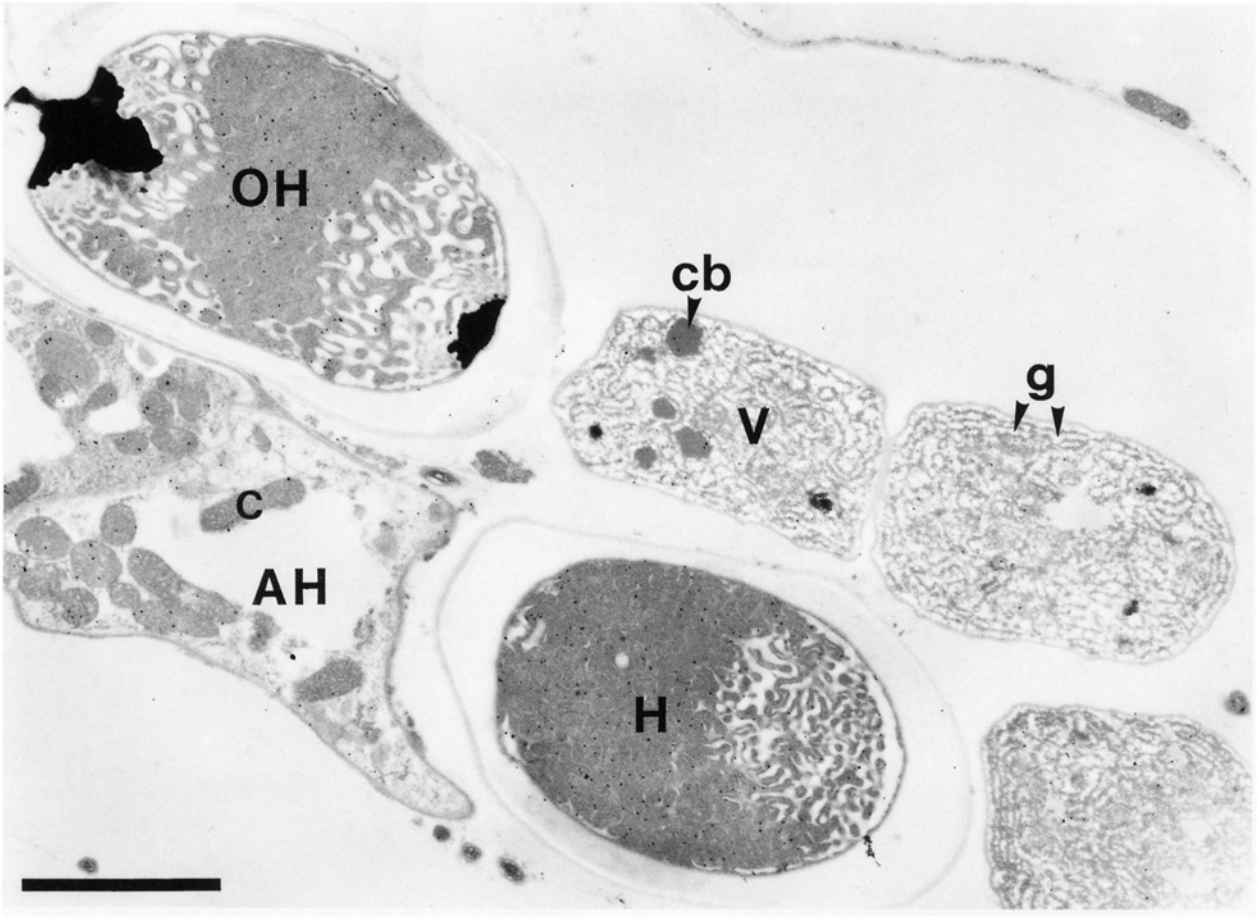

Fig. 4. Localization of 32-kDa protein in cells present within a thin-section through an older *Azolla* leaf cavity (approx. leaf No. 8 from the apical meristem) using Ab 2. Little 32-kDa protein is detectable within vegetative cells (*V*) having large accumulations of glycogen (*g*). However, gold particles are readily apparent in mature (*H*) and older (*OH*) heterocysts, and in chloroplasts (*c*) of *Azolla* hair cells (*AH*). Note that the honeycomb structures of heterocysts contain less 32-kDa protein than other regions of the cell. Immunogold particles are occasionally seen on carboxysomes (*cb*). Bar = 2 μm; × 13300

tein, Ab 1 (Mei et al. 1989), are shown in Fig. 1. As expected, the 32-kDa protein was localized on thylakoid membranes within vegetative cells. In addition, immunologically recognizable 32-kDa protein was found on membranes within heterocysts, which are easily distinguished from vegetative cells by the presence of extra cell-wall layers. Both heterocysts shown in Fig. 1 exhibit ultrastructural characteristics of mature heterocysts (Braun-Howland et al. 1989). Immunogold label within *Azolla* chloroplasts, and its lack within non-photosynthetic bacteria, provided positive and negative internal controls, respectively, for these studies.

Because the 32-kDa protein is integrated within membranes, the amount of protein detected in a cell was dependent upon the orientation of the protein in the membrane with respect to the plane of sectioning. The number of immunogold particles within individual cells and chloroplasts frequently varied considerably between serial sections (data not shown). Precise quantitative analyses of the amount of 32-kDa protein in different cell types is therefore not feasible using immunolabeling of thin sections and TEM.

To determine whether both 32-kDa protein and dinitrogenase reductase were present concomitantly within heterocysts, serial sections were immunolabeled using primary antibodies directed against one or the other of these two proteins. The results of immunogold labeling with antibodies directed against dinitrogenase reductase are shown in Fig. 2A. Dinitrogenase reductase was detected in both mature and old heterocysts, but not in vegetative cyanobacterial cells or *Azolla* hair cells. Labeling of serial sections through the same group of cells with 32-kDa antiserum (Fig. 2B) demonstrated that heterocysts containing dinitrogenase reductase also contained the 32-kDa protein. As expected, 32-kDa protein was also observed in vegetative cyanobacterial cells and chloroplasts of *Azolla* hair cells, while neither protein was detected in symbiotic bacteria (Fig. 2A, B).

Immunolabeling results using a different antiserum (Ab 2), directed against a peptide within a hydrophilic transmembrane loop of the 32-kDa protein (Qb-4; Sayre et al. 1986), are presented in Figs. 3 and 4. In general, the labeling pattern obtained with this antiserum was consistent with that obtained using Ab 1 (compare

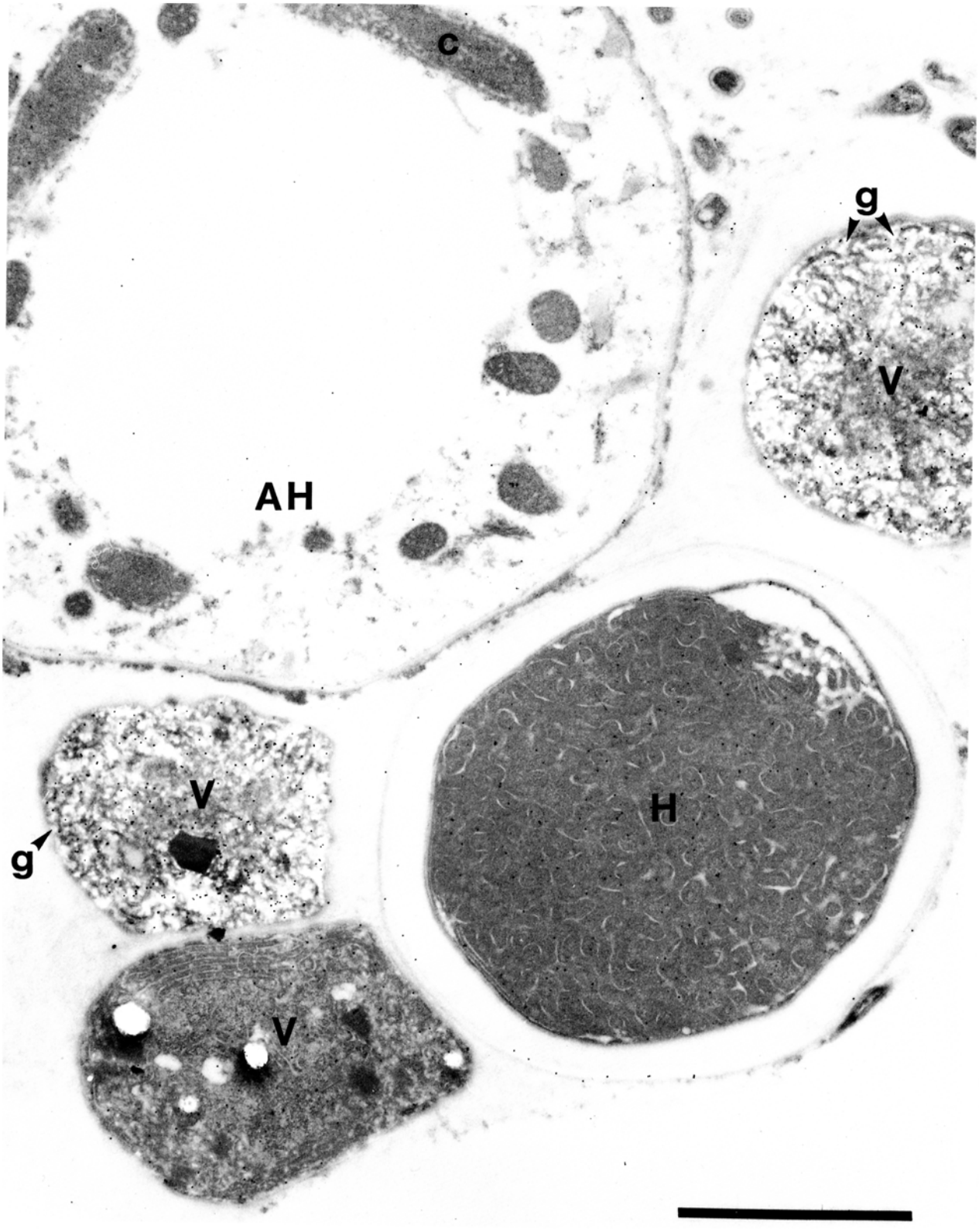

Fig. 5. Immunolabeling of 32-kDa protein in vegetative cells (*V*), a mature heterocyst (*H*) and *Azolla* hair cell (*AH*) chloroplasts (*c*) present within an *A. caroliniana* leaf cavity using Ab 3, specific for the carboxyl-terminal two-thirds of the protein. Vegetative cells containing large amounts of glycogen (*g*) contain more label than vegetative cells containing little glycogen or heterocysts. Bar = 2 μm; × 20000

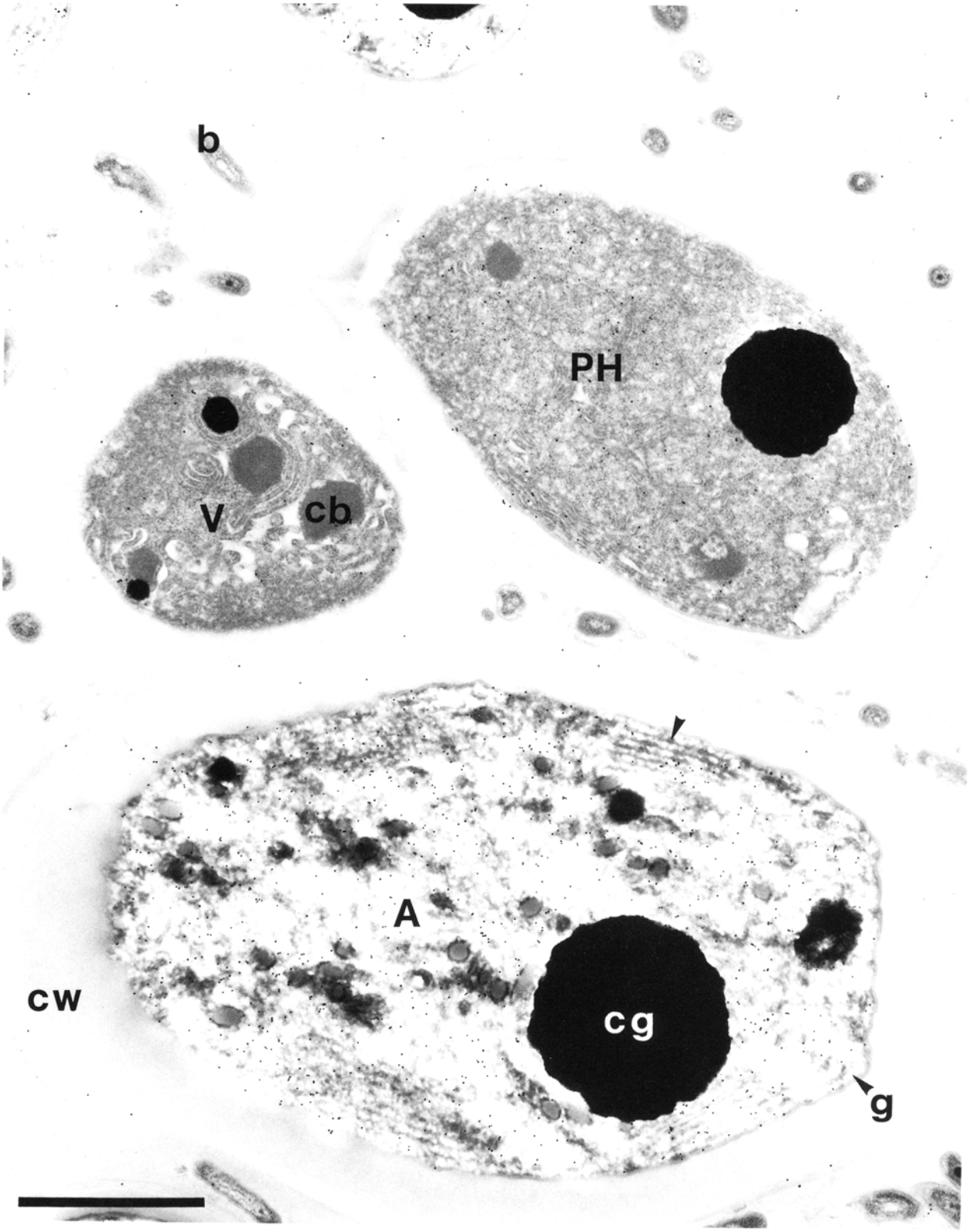

Fig. 6. Immunogold labeling of 32-kDa protein within different cell types of the *A. caroliniana* cyanobionts using Ab 3. Akinetes (*A*) are distinguished by large accumulations of glycogen (*g*) and cyanophycin granules (*cg*) and the presence of thickened extra cell-wall layers (*cw*). Phycobilisomes (*arrowhead*) are evident on rearranged thylakoid membranes. Both vegetative cells (*V*) and akinetes contain carboxysomes (*cb*), although they are not evident in this particular section through an akinete. More 32-kDa protein was detected in akinetes than in young vegetative cells, proheterocysts (*PH*) and mature heterocysts. Labeling in symbiotic bacteria (*b*) was not above biological background. Bar = 2 μm; × 16000

Figs. 1 and 2B with 3). Figure 3A shows the 32-kDa protein localized on both granal and stromal membranes of *Azolla* chloroplasts. The presence of 32-kDa protein in heterocysts at different stages of the differentiation process is shown in Fig. 3B and C. Immunolabeling of a proheterocyst, which is easily distinguished from vegetative cells by the presence of extra cell-wall layers, fewer inclusion bodies, a narrowed neck containing polar plugs and rather diffuse membranes, is shown in Fig. 3B. In old heterocysts, which are ultrastructurally distinguished by a high degree of membrane vesiculation at the poles, 32-kDa protein was also detected (Fig. 3C). As was observed for dinitrogenase reductase (Braun-Howland et al. 1988), less 32-kDa protein was found in the honeycomb-membrane configurations that are found within the polar regions of mature and older heterocysts, than in the central cellular regions. Occurrence of the 32-kDa protein in heterocysts is therefore not restricted to a particular stage of the differentiation process.

In the *A. caroliniana* cyanobionts, both older vegetative cells and akinetes contained large accumulations of glycogen and cyanophycin. However, akinetes were easily distinguished from vegetative cells by their larger size and by the presence of additional cell-wall layers. A developing akinete, immunolabeled with Ab 2 to localize the 32-kDa protein, is shown in Fig. 3D. Interestingly, very little 32-kDa protein was detected in developing akinetes of the *A. caroliniana* cyanobionts when either Ab 2 or Ab 1 (data not shown) was used as the primary antibody. Furthermore, when either antiserum was used, little label was observed within any cyanobacterial cell type containing large glycogen deposits, including mature akinetes and older vegetative cells. This phenomenon is illustrated in Fig. 4 (see also Fig. 2B), which shows relative amounts of 32-kDa protein detected in older cyanobacterial vegetative cells, heterocysts and *Azolla* chloroplasts. Whereas the amount of 32-kDa protein within older heterocysts approximates that found within younger heterocysts (except within the heavily vesiculated honeycomb configurations), little or no 32-kDa protein was detected in either older vegetative cells or bacteria. Although precise quantitative analysis was not possible, cells containing large glycogen accumulations clearly contained less immunogold label than did younger vegetative cells or heterocysts. When Ab 1 or Ab 2 was employed, gold particles were frequently localized on cyanobacterial carboxysomes (see Figs. 3C, 4). This apparently inclusion-specific artifact may result from the close temporal association of carboxysomes and thylakoid membranes.

In contrast to Ab 1 and Ab 2, which are site-directed antisera raised against small, hydrophilic epitopes of the 32-kDa protein, the third antiserum used in these studies (Ab 3), was a polyclonal antiserum raised against the carboxyl-terminal two-thirds of the *Amaranthus hybridus* 32-kDa protein (Ohad et al. 1985). The results of immunolabeling studies using this antiserum were consistent with those described above in that the 32-kDa protein was localized on membranes within young vegetative cells and heterocysts of the cyanobionts (Figs. 5, 6), as well as in *Azolla* chloroplasts (Fig. 5). However, major differences in labeling patterns, dependent upon the primary antiserum employed, were also evident. Whereas little or no 32-kDa protein was detected in cells containing large glycogen deposits when either Ab 1 or Ab 2 was employed as primary antiserum, considerably more label was found in these cell types when they were immunolabeled with Ab 3. As illustrated in Fig. 5, vegetative cells containing greater amounts of glycogen clearly contained more gold particles than did either heterocysts or young vegetative cells. Results of immunolabeling with Ab 3 in a mature akinete are presented in Fig. 6. As was observed in older vegetative cells, relatively greater amounts of 32-kDa protein were detected in mature akinetes than in younger vegetative cells and heterocysts of all ages.

Discussion

Our results demonstrate that immunologically recognizable 32-kDa protein is found within all cell types of the *A. caroliniana* cyanobionts. Because cyanobionts within the *A. caroliniana* association have been shown to fix CO_2 by way of the Calvin cycle (see Kaplan and Peters 1988), the localization of 32-kDa protein on thylakoid membranes of vegetative cells was not surprising. However, we also find that the 32-kDa protein is present within cyanobacterial heterocysts, which apparently lack an active PSII.

Although numerous authors have concluded that heterocysts of free-living cyanobacteria lack PSII activity (Thomas 1970; Bradley and Carr 1971; Donze et al. 1972; Tel-Or and Stewart 1977; Alberte et al. 1980; Almon and Böhme 1980; Peterson et al. 1981), heterocysts have been shown to contain several components associated with PSII-mediated electron transfer (Tel-Or and Stewart 1977; Almon and Böhme 1980). The function of PSII components in heterocysts is unknown. Newton and Tyler (1987) have proposed the intriguing possibility that PSII, particularly the 32-kDa protein, may play a role in cyanobacterial ammonia metabolism. If the 32-kDa protein does function in the metabolism of fixed nitrogen, this may provide an explanation for the presence of the 32-kDa protein within heterocysts of the *A. caroliniana* cyanobionts.

It should not be inferred from our results that the 32-kDa protein is integrated into a functional (O_2-generating) PSII apparatus within heterocysts of the cyanobionts. Indeed, the concomitant presence of dinitrogenase reductase and the 32-kDa protein in mature heterocysts (Fig. 2) would argue against this; both in-vivo (Rippka and Stanier 1978) and in-vitro (Fay and Cox 1967) studies have demonstrated that nitrogenase is irreversibly inactivated by exposure to oxygen.

Whether the presence of 32-kDa protein in heterocysts is confined to *Azolla* cyanobionts or if it is universally present within cyanobacterial heterocysts is not known. In addition to having an altered spacing pattern, heterocysts of the *Azolla* cyanobionts differ substantially

from those of most free-living cyanobacterial species (see Adams and Carr 1981) in several respects, including the metabolism of fixed nitrogen (Newton and Cavins 1976; Ray et al. 1978; Orr and Haselkorn 1982) and the retention of phycobiliproteins (Becking and Donze 1981) during heterocyst differentiation.

Because several lines of evidence indicate that PSII is structurally associated with phycobilisomes (see Mörschel and Rheil 1987), and phycobilisomes are clearly discernable on akinete "thylakoid" membranes (Fig. 6), the presence of 32-kDa protein within akinetes was not unexpected. Photosystem-II activity has been shown to be necessary for efficient germination of akinetes in some cyanobacterial species (see Herdman 1987).

Although differences in the quantity and ultrastructure of membranes in cyanobacterial cell types precluded precise quantitative analysis, certain labeling patterns were evident. Whereas all three antisera employed in the present study detected 32-kDa protein within young vegetative cells, heterocysts and *Azolla* chloroplasts, the detection of 32-kDa protein within akinetes and older vegetative cells was dependent upon the antibody employed (compare Figs. 4 and 6). We suggest that variations in labeling were a consequence of differential accessibility of antigenic sites against which the different 32-kDa antisera were prepared. In particular, the apparent lack of 32-kDa protein in akinetes and older vegetative cells when Ab 1 or Ab 2 were employed may have resulted from masking of the small hydrophilic antigenic sites (against which Ab 1 and Ab2 were raised), possibly by large accumulations of glycogen within these cells.

In *Anabaena variabilis*, the average half-life of total messenger RNA is less than 20 min (Leach and Carr 1974; Wealand et al. 1989). Furthermore, a light-dependent rapid turnover of the 32-kDa protein has been demonstrated in *Synechococcus* PCC7942 (Goloubinoff et al. 1988) and in chloroplasts of higher plants (see Mattoo et al. 1981). We therefore assume that the 32-kDa protein is actively transcribed and translated in both heterocysts and akinetes, as well as vegetative cells, of the *A. caroliniana* cyanobionts.

Our results support the proposal that *psbA* genes are transcribed in heterocysts of *Azolla* cyanobionts. The results of the present study also indicate that transcription of *psb*A genes in both heterocysts and akinetes contributed to the increased levels of *psb*A transcripts observed in symbiotic versus free-living *Anabaena* (Nierzwicki-Bauer and Haselkorn 1986). It should also be kept in mind that rates of transcription, translation, and-or protein turnover are likely to differ between vegetative cells, heterocysts and akinetes (see Herdman 1987). If these rates differ in the *A. caroliniana* cyanobionts, then the occurrence of 32-kDa protein in heterocysts and akinetes raises the possibility that growth conditions, which govern the differentiation of both cell types, affected the outcome of comparative RNA quantitation studies.

Last, in higher plants, the chloroplast genome contains one copy of the *psb*A gene, encoding the 32-kDa protein (Zurawski et al. 1982). However, multiple *psb*A gene copies have been identified in *Anacystis nidulans* R2 (Mulligan et al. 1984; Golden and Haselkorn 1985), *Fremyella diplosiphon*, *Nostoc* sp. MAC (Mulligan et al. 1984), *Anabaena* 7120 (Curtis and Haselkorn 1984) and cyanobionts of *A. caroliniana* (Nierzwicki-Bauer and Haselkorn 1986). Recent studies have shown that expression of individual *psb*A gene-family members is dependent upon light in unicellular cyanobacteria of *Synechococcus* spp. (Lönneborg et al. 1988; Schaefer and Golden 1989). However, differential *psb*A gene expression has not yet been examined in filamentous cyanobacteria capable of differentiating heterocysts and-or akinetes. We hypothesize that in the cyanobionts of *A. caroliniana*, there is differential expression of *psb*A gene family members in different cyanobacterial cell types. This is currently under investigation in our laboratory.

This work was supported by Grant No. 1 RO1 GM37051-01 awarded by the National Institute of General Medical Sciences to S.A.N.-B. We are grateful to Drs. Harry Roy and Michael Hanna for helpful discussions during the course of this work. The contributions of Mrs. Mary Ann Sessions to the final draft of the manuscript are also gratefully acknowledged. Special thanks are due to Drs. J. Oelze, L. McIntosh and R. Sayre for providing the antisera used in these studies.

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Received 5 September; accepted 10 October 1989