

Chapter 2

Chloroplast Proteins

General Structural Features of Chloroplasts

Chloroplasts are the most complex organelle in the plant cell. The chloroplast is encircled by a double membrane structure known as the envelope, the site where cytoplasmic proteins are processed into their final forms. The inner chloroplast membrane may be invaginated in a series of reticulations, the peripheral reticulum of C4 plant chloroplasts. Less extensive invaginations occur commonly in other plants. The stroma area contains the enzymes for photosynthetic carbon fixation as well as numerous other enzymes and 70S ribosomes. The thylakoid membranes are the site of photosynthetic light reactions and certain other unique proteins. The proteins exist *in vivo* as complexes of proteins and photosynthetic pigments. Because of this complexity in both structure and the number of proteins present, techniques have been developed to fractionate each part of the chloroplast, which has in some ways supplanted the role of immunocytochemistry in determining the distribution of proteins within the chloroplast. These methods, in some cases, do rely upon fairly drastic treatment of the membranes, making such localizations equivocal. Immunocytochemistry allows the localization of the protein *in situ* rather than relying upon fractionation procedures.

There are several primary areas where immunocytochemistry has impacted chloroplast studies:

- (1) The tissue and subcellular distribution of the primary enzyme of carbon fixation, ribulose biphosphate carboxylase/oxygenase (RuBisCo) in both C3 and C4 plants, lower land plants and algae.
- (2) The distribution of the photosynthetic complexes within the thylakoid membranes in both algae and higher plants.
- (3) The development of chloroplast from proplastids.
- (4) Determining the sites for chloroplast protein entry into the chloroplast.

Distribution of RuBisCo

In most C4 plants, the chloroplasts which are involved in CO₂ fixation (in bundle sheath cells) are separated physically from the chloroplasts that are generating O₂ as a consequence of water-splitting due to photosynthetic electron transport (in mesophyll cells). This separation allows for chloroplasts with relatively low O₂ concentrations to fix CO₂, eliminating the energetically costly photorespiration from occurring to any extent. It is possible to isolate mesophyll and bundle sheath chloroplast by differential grinding techniques as the mesophyll cell chloroplasts come out with relatively weak grinding conditions leaving the bundle sheath cells intact (Vaughn and Duke 1981a). Grinding techniques such as this give a strong indication of the distribution but are always fraught with the problems of contamination of one fraction into another. Immunocytochemical techniques however clearly showed the same dichotomy of RuBisCo distribution: RuBisCo was restricted to the bundle sheath cell chloroplasts (Hattersley et al. 1977) and the first enzyme of CO₂ fixation in C4 plants, PEP carboxylase, was found in the cytoplasm of mesophyll cells (Madhavan and Smith 1982, 1984).

Guard cells are generally the only photosynthetic cells in the epidermis of plant cells and these cells are widely distributed in a large predominance of non-guard cells. Although techniques have been developed for their isolation, the problem with even small contaminations of mesophyll cells is even more of a problem. This is where cytochemical and immunocytochemical protocols can answer questions directly. Vaughn (1987) and Vaughn and Vaughan (1988) revealed that guard cells reacted strongly but not as strongly as those with mesophyll cells. Immunogold quantification revealed about 1/6th of the density of the labeling of RuBisCo in the stroma of the guard cell chloroplast. Data such as these would've been difficult to obtain using traditional fractionation protocols. To further substantiate these results a series of variegated chimera geraniums was used to prove these data both immunocytochemically and via standard immunochemical protocols. In these variegated chimeras, the epidermal layer is genetically green but the mesophyll cells in the white tissue lack 70S ribosomes and are thus incapable of producing the large subunit of RuBisCo. The white plastids did not label with anti-RuBisCo antibodies although the guard cell chloroplasts did. Similarly, Western blots of the white tissue had a band at 55kD that matched the band from green tissues, indicating that the source of the immunoreactive band on the Western blots was due to the guard cell chloroplast RuBisCo.

Not all plastids are involved in photosynthesis. Immunocytochemical studies of chloroplasts of *Capsicum* revealed that green plastids characteristic of unripe fruit had strong labeling of RuBisCo, whereas the mature red fruits had much reduced labeling (Cheniclet et al. 1988).

Pyrenoids in algae and hornworts were believed to be accumulations of RuBisCo protein and immunocytochemistry revealed that these conclusions were correct (Lacoste-Royal and Gibbs 1985; McKay and Gibbs 1989; Vaughn et al. 1988). Although it might have been possible to isolate enough algal cells to perform a

biochemical isolation of the pyrenoid, this would've been impossible for the relatively slow-growing hornworts. Moreover, in the hornworts, the pyrenoids are often in a multiple pyrenoid formation and have inclusions (pyrenoglobuli) that are non-reactive (Vaughn et al. 1990). A further question was whether the RuBisCo in the pyrenoids represented active RuBisCo or merely a stored form. A low reactivity in the stroma of some algae indicated that there may be two pools, active and inactive, and perhaps the stromal form represented the active component. In hornworts, essentially no labeling was detected in the non-pyrenoid areas, however. Before RuBisCo is active it complexes with RuBisCo activase. Localization of RuBisCo activase in both algae and hornwort chloroplasts reveals a predominant localization of the RuBisCo activase in the pyrenoid along with the RuBisCo (McKay et al. 1991). Thus, the pyrenoid RuBisCo represents a functioning RuBisCo. The pyrenoid is not the site of all Calvin cycle enzymes, however. McKay and Gibbs (1991) showed that phosphoribulokinase, the next enzyme in the Calvin cycle pathway, was clearly stromal.

We performed almost all of our work with sectioned material. RuBisCo is the most abundant protein and reacts well even after fixation and embedding. Chloroplasts are highly autofluorescent, which makes eliminating this fluorescence source as a "specific" immunolabelling. In these cases, the use of immunogold or immunogold silver lead to unequivocal labeling. We utilized relatively high concentrations of primary antibody as we found that labeling levels increased dramatically up to about a 1:20 dilution of the primary rabbit or goat antibodies. Although this dilution seems high, the amount of RuBisCo present in the chloroplast is also high and even these relatively high levels of primary antibody resulted in no significant labeling of other structure (e.g., Vaughn et al. 1988). This same logic was used in rationalizing the use of high concentration of both monoclonal and polyclonal antibodies to cell wall components as even more concentrated solutions (to "neat" or undiluted) as the presence of so many reactive epitopes were present in the section, antibody excess was not reached.

Photosystem, Light-Harvesting Complexes and Other Thylakoid Proteins

The distribution of photosystem complexes was a major topic of discussion in the 1980's and immunocytochemical studies were an important part of these studies. Thylakoids may be easily isolated from chloroplasts and these may be further fractionated into stroma lamellae (unstacked) and grana (stacked) thylakoid membrane, using a series of detergents and fractionation techniques. A potential third class of membrane surfaces are the so-called grana end membranes that are areas of stacked thylakoid but are exposed to the stroma.

The first immunocytochemical localization of a thylakoid was the localization of the chloroplast coupling factor (Miller and Staehelin 1976). This study utilized isolated thylakoid membranes, which were labeled prior to post-fixation so that

none of the problems associated with loss of antigenicity during the processing of samples through plastic could be encountered. These studies revealed that the CF1 was associated with the unstacked areas of lamellae, although there might have been the question of penetration of the rather large antibody molecules into the stacked regions of thylakoids. CF1 has a distinct shape and with the proper staining conditions, CF1 may be seen in both thin sections and isolated CF1 particles that confirmed the ideas that CF1 was limited to the unstacked areas. Moreover, studies by Allred and Staehelin (1985), Vallon et al. (1986), Lax and Vaughn (1991) and Pettigrew and Vaughn (1998) revealed the same sort of localizations in thin sections, where there would be no impedance to the access of the antibody to the stacked thylakoid areas.

Another early immunocytochemistry study used the peroxidase anti-peroxidase technique to determine the distribution of the photosystem 1 chlorophyll a protein (Vaughn et al. 1983; Vaughn 1986). Sections of material embedded in resin were reacted with affinity-purified serum to the P700 complex raised in rabbits. The antibody recognized the apoprotein of this complex (~ 67 kD) on Western blots and no other associated protein. The peroxidase anti-peroxidase (PAP) technique is very sensitive and initial studies revealed that the protein was present throughout the chloroplast membranes (Vaughn et al. 1983) although further dilution of this serum revealed a differential labeling of the stroma lamellae and grana end membranes (Vaughn 1987). The peroxidase anti-peroxidase complex reacts with the diamino benzidine (DAB) which forms an osmiophilic polymer. Because the reaction is not particulate, the best one can attain is a relative distribution of the protein of interest. One of the advantages is, however, that the increased sensitivity of this technique compared to the unamplified techniques such as immunogold. When antiserum is rare and exact quantification is not needed, PAP is still a useful protocol.

Numerous studies have shown that the PSII-related proteins are predominantly associated with stacked membranes (Vallon et al. 1986; Lax and Vaughn 1991; Pettigrew and Vaughn 1998). The more limited stacked membranes are also the sites of PSII activity and protein in the algae as well (Vallon et al. 1986; McKay et al. 1992; Cunningham et al. 1991; Vesik et al. 1992). Interestingly, even thylakoids that run through the pyrenoid area have PSII proteins present (McKay et al. 1992). Similar observations were made on the thylakoid membranes adjacent to the pyrenoids in hornworts (Vaughn, unpublished). The cytochrome b6/f complex appears to be associated with both stacked and unstacked membranes (Olive et al. 1986; Pettigrew and Vaughn 1998).

Polyphenol oxidase (PPO) is one of the most enigmatic of the chloroplast proteins. Although it was known to be a chloroplast protein since the late 1940's, its function in the chloroplast and its sub-chloroplast distribution has been the matter of debate (Vaughn et al. 1988). Cytochemical studies use the oxidation of DOPA to a DOPA-quinone polymer to detect PPO. The osmiophilic DOPA-quinone product is found along all of the thylakoids (Vaughn and Duke 1981a), suggesting that PPO is uniformly distributed along the thylakoids. However, the DOPA-quinone product is not completely insoluble and probably is able to move along the thylakoids before being rendered insoluble by osmium post-fixation.

Lax and Vaughn (1991) used a combination of chloroplast fractionation and immunocytochemical procedures that showed that PPO is actually a portion of the PSII complex and is preferentially associated with stacked and not un-stacked membranes. These compared with the distribution of PSII associated proteins, such as the light-harvesting complex of PSII and not the distribution of the P700 chl a protein or CF1. These data were consistent with a previous study of the C4 plant sorghum, where PPO was associated with the PSII-containing mesophyll chloroplasts but was absent in the PSII-deficient bundle sheath chloroplasts (Vaughn and Duke 1981a).

PPO is also found in non-green plastids. In these plastids, the PPO is generally present in a membrane-bound body, like a highly distended thylakoid (Vaughn et al. 1981). Because these plastids are non-green, it was assumed that the function of PPO in these plastids was related to the ability of this enzyme to react with phenols and convert them into highly reactive quinines that could be used in plant defense. This was also supported by the distribution of these non-green plastids in epidermal cells which would be the first site of invasion of insect predators or fungi. These same cells were also the site of the highest concentrations of phenolic compounds, present in the vacuole. Damage caused by insect predation would allow the compartmentalized phenolics to mix freely with the membrane-bound PPO, creating large amounts of a brown sticky polymer. An extreme example of this occurs in some *Solanum* species, which contain a high density of trichomes on the leaf surface. Each of these trichomes contains many plastids with bodies that contain high concentrations of PPO and a high concentration of phenolics within the vacuole. The large amounts of polymer produced actually entrap aphids and other small insects.

PPO is a nuclear-encoded protein that is transported into the chloroplast. Mutants without 70S ribosomes still can accumulate PPO (Vaughn et al. 1981). However, the natural product tentoxin can cause a chlorosis that produces yellow plants. These plastids lack an active PPO as measured by DOPA oxidation and cytochemical techniques. However, antiserum to PPO reacts with the envelope membranes of these yellow plastids from tentoxin-treated plants (Vaughn and Duke 1984) and an inactive form of PPO accumulates as a precursor molecule along the chloroplast envelope. These data were used to show that tentoxin's primary effect is on the incorporation of nuclear-encoded chloroplast proteins into the chloroplast, not the inhibition of chloroplast coupling factor, as previously reported. Moreover, chloroplast mutants devoid of CF1 are also strongly affected by tentoxin (Lax and Vaughn 1986).

Chloroplast Development

Chloroplast development occurs from the very under-developed proplastids that are found in the meristem and rapidly develop into chloroplasts that are completely functional. Unfortunately, isolating the plastids from meristems and very small

leaves is difficult or impossible because of the ability to obtain sufficient material and develop techniques to isolate the pure organelles. Instead, studies of chloroplast development have relied upon the conversion of etioplasts (from plants grown in darkness) to chloroplasts so that sufficient numbers of plastids may be obtained. This process of etioplast to chloroplast is not the normal pathway for chloroplast development and it is likely that the developmental pathways involved are different than the proplastid to chloroplast conversion.

To investigate some of the developmental processes, Pettigrew and Vaughn (1998) followed the progression of chloroplast development from the smallest leaves that could be monitored for chlorophyll fluorescence and CO₂ fixation characteristics to fully formed leaves. To study this development, sections from five developmental stages were probed with 18 different antibodies that recognize enzymes of chloroplast metabolism and thylakoid proteins plus one enzyme of photorespiration. Quantitative analysis was used to calculate the labeling density of each of the antibodies. Stromal enzyme densities were calculated by determining the density of gold particles/ μm^2 , whereas the thylakoid proteins were calculated on a density of gold/linear micron of thylakoid membrane. Although the densities of labeling could not be compared between different proteins because of differences in the ability of the serum to recognize the proteins in the section, the increase in density and the first appearance of the protein could be determined. These studies established something quite different for chloroplast development than the conversion of chloroplasts from etioplasts to chloroplasts. In those etioplast to chloroplast studies, PSI reactions were observed first and the thylakoids were unstacked. However, in normal chloroplast development highly-stacked membranes and associated PSII activity were observed first and unstacked stroma lamellae and associated CF1 and PSI proteins occurring secondarily. In a companion study, Vaughn and Pettigrew (unpublished) observed meristems and the first primordial that were too small to be sampled for fluorescence or CO₂ fixation ability. Although no chloroplast proteins were observed in the meristematic cells, even very young primordial chloroplasts had RuBisCo and the small stacked membranes had LHC labeling. These tissues were too tiny to be used for biochemical studies or photosynthetic measurements but indicate just how quickly the meristematic proplastids become differentiated into chloroplasts.

Sites of Plastid Protein Uptake

One of the biggest questions in chloroplast development centered on the movement of chloroplast proteins into the chloroplast and how proteins are able to cross the two chloroplast envelope membranes. A very interesting approach to this question was applied by Pain et al. (1988). These authors assumed that antibodies raised to nuclear-encoded chloroplast proteins might produce antibody classes that resemble the natural receptors that recognize these proteins at the chloroplast envelope. Accordingly, the authors raised an antibody to the small subunit of

RuBisCo, isolated these rabbit antibodies and raised anti-idiotypic antibodies (Knigge et al. 1989) in rabbit. When this serum was used to probe Western blots, a 30kD protein reacted strongly and this antibody could inhibit SSU uptake into the chloroplast. When these antibodies were used on preparations of isolated chloroplasts, the antibodies labeled only sites where there was fusion of the two envelope membranes, a site that would make crossing only one membrane a requirement rather than two for uptake of nuclear-encoded proteins. The SSU is a component of the most abundant chloroplast protein and is strictly stromal. It would be interesting to perform these same series of anti-idiotypic antibody production with proteins such as the light-harvesting complex of PSII or even the portion of the apoprotein that is cleaved upon entry of the apoprotein into the chloroplast.

The herbicide glyphosate inhibits the chloroplast enzyme EPSP synthase, an enzyme of aromatic amino acid biosynthesis. When plants are treated with glyphosate, a precursor form of EPSP synthase accumulates. Interestingly, when antibodies to EPSP synthase are used on thin sections of plants or cultures pre-treated with glyphosate the label occurs uniformly around the chloroplast, not just in areas where membrane appression occurs (Smeda and Vaughn 1997). These data may mean that several sites along the envelope are able to recognize and be involved with incorporation of the nuclear-encoded protein into the chloroplast.

Chloroplast Lipids

Two of the earliest immune-labeling studies involve the localization of the unique chloroplast lipids, galactolipids and sulfolipids (Billecocq 1972; 1975). In these studies, lipids were attached to BSA and injected into rabbits for antibody production. Chloroplast membranes were incubated in enzyme-coupled antibody and the peroxidase reaction was performed using DAB. Labeling of the membrane revealed a strong reaction with both of these antibodies.

The Enigma of Nitrate Reductase

Nitrate reductase is one of the most enigmatic proteins in terms of localizations. Many of the cell fractionation studies and some of the immunocytochemical localization studies have indicated that nitrate reductase was a chloroplast enzyme (Kamachi et al. 1987). Unfortunately, nitrate reductase is present in the cell in relatively low quantities and is extremely sensitive to proteolysis. A cytochemical localization of the nitrite accumulated as measured by an azo dye, revealed a cytoplasmic distribution of the reaction product in tissue with elevated levels of nitrate reductase (Vaughn and Duke 1981b). To investigate this further, Vaughn and Campbell (1988) examined maize leaves using a highly purified nitrate reductase antiserum in maize leaves. Maize leaves are unique in that nitrate

reductase is restricted in distribution to the mesophyll cells based upon results from fractionation protocols, thus providing an internal control for the experiment. To improve the conditions for labeling, a pre-embedding labeling was attempted so that less of the nitrate reductase would be lost due to denaturation during the embedding protocols. Label was restricted to the mesophyll cells and no reaction was noted in chloroplasts even when the ends of the chloroplast were clearly visible as being opened and accessible to the antibodies. These data indicate that other studies localizing nitrate reductase to plastids are probably the result of non-specific antibody binding either to RuBisCo or other chloroplast proteins. The example of nitrate reductase being localized to the pyrenoid, a site of RuBisCo accumulation, in the green algae is an example of non-specific antibody sticking to RuBisCo and not a localization of nitrate reductase (Lopez-Ruiz et al. 1985).

Summary

Chloroplasts are an amazingly complex plant organelle containing very distinct sets of membranes and stromal space. Immunocytochemical studies have revealed many important aspects about both tissue and sub-chloroplast distributions of proteins and other epitopes. RuBisCo distribution in C4 plants being restricted to the bundle sheath cells and a low (1/6th of mesophyll chloroplasts), but detectable, level of RuBisCo were also found in guard cells. A number of thylakoid proteins have been localized, with PSII proteins concentrated in the stacked membranes and PSI proteins and chloroplast coupling factor predominantly or exclusively in the unstacked membranes. Developmental studies on the normal development of chloroplasts from apical proplastids revealed that stacked membranes actually occur even in the youngest thylakoid membranes and actually occur before PSI. Although some studies indicate that at least the uptake of the SSU of RuBisCo occurs in appressed envelope membranes, other studies are needed to determine if this site holds for all chloroplast proteins.

References

- Allred DR, Staehelin LA (1985) Lateral distribution of the cytochrome b6/f and CF0/CF1 complexes of thylakoid membranes. *Plant Physiol* 78:199–202
- Billecocq A (1975) Structure des membranes biologiques: localization du sulfoquinovosyldiglyceride dans les diverses membranes des chloroplasts au moyen des anticorps spécifiques. *Ann Immunol* 126C:337–352
- Billecocq A, Douce R, Faure M (1972) Structure des membranes biologiques. localization des galactosyldiglycerides dans au moyen des anticorps spécifiques. *C R Acad Sci Paris* 275D:1135–1137
- Cheniclet C, Suire C, Carde JP (1988) Immunocytochemical detection of ribulose biphosphate carboxylase in *Capsicum* plastids. *Biol Cell* 62:289–292

- Cunningham FX, Mustardy L, Gantt E (1991) Irradiance effects on thylakoid membranes of the red alga *Porphyridium cruentum*. An immunocytochemical study. *Plant Cell Physiol* 32:419–426
- Hattersley PW, Watson L, Osmond CB (1977) In situ immunofluorescent labeling of ribulose-1,5-bisphosphate carboxylase in leaves of C3 and C4 plants. *Aust J Plant Physiol* 4:523–539
- Kamachi K, Amemiya Y, Ogura N, Nakagawa H (1987) Immuno-gold localization of nitrate reductase in spinach (*Spinacea oleracea*) leaves. *Plant Cell Physiol* 28:333–338
- Knigge KM, Piekut DT, Abood LG, Joseph SA, Michael GJ, Xin I, Berlove DJ (1989) Immunocytochemistry of receptors using anti-idiotypic antibodies. *Meth Enzymol* 178: 212–221
- Lacoste-Royal G, Gibbs SP (1985) *Ochromonas* mitochondria contain a specific chloroplast protein. *Proc Natl Acad Sci U S A* 82:1456–1459
- Lax AR, Vaughn KC (1986) Lack of correlation between effects of tentoxin on chloroplast coupling factor and chloroplast ultrastructure. *Physiol Plant* 66:384–391
- Lax AR, Vaughn KC (1991) Polyphenol oxidase co-distributes with photosystem II. *Plant Physiol* 96:26–31
- Lopez-Ruiz A, Verbelen JP, Roldan JM, Diez J (1985) Nitrate reductase of green algae is localized in the pyrenoid. *Plant Physiol* 79:1006–1010
- Madhavan S, Smith BN (1982) Localization of ribulose bisphosphate carboxylase in the guard cells by an indirect, immunofluorescence technique. *Plant Physiol* 69:273–277
- Madhavan S, Smith BN (1984) Phosphoenoyl pyruvate carboxylase in guard cells of several species by an indirect immunofluorescence technique. *Protoplasma* 122:157–161
- McKay RML, Gibbs SP (1989) Immunocytochemical localization of ribulose 1,5-bisphosphate carboxylase/oxygenase in light-limited and light-saturated cells of *Chlorella pyrenoidosa*. *Protoplasma* 149:31–37
- McKay RML, Gibbs SP (1991) Immunocytochemical localization of phosphoribulokinase in microalgae. *Bot Acta* 104:367–373
- McKay RML, Gibbs SP, Vaughn KC (1991) Rubisco activase is present in the pyrenoid of green algae. *Protoplasma* 162:38–45
- McKay RML, Lichtle C, Gibbs SP (1992) Immunocytochemical characterization of the intrapyrenoid thylakoids of cryptomonads. *J Phycol* 28:64–68
- Miller KR, Staehelin LA (1976) Analysis of the thylakoid outer surface: coupling factor is limited to unstacked membrane regions. *J Cell Biol* 68:30–47
- Olive J, Vallon O, Wollman FA, Recouvreur M, Bennoun P (1986) Studies on the cytochrome b6/f complex. II. Localization of the complex in the thylakoid membranes from spinach and *Chlamydomonas reinhardtii* by immunocytochemistry and freeze-fracture analysis of b6/f mutants. *Biochim Biophys Acta* 851:239–248
- Pain D, Kanwar YS, Blobel G (1988) Identification of a receptor for protein import into chloroplasts and its localization to envelope contact zones. *Nature* 331:232–237
- Pettigrew WT, Vaughn KC (1998) Physiological, structural and immunological characterization of leaf and chloroplast development in cotton. *Protoplasma* 202:23–37
- Smeda RJ, Vaughn KC (1997) Mechanisms of resistance to herbicides. *Chem Plant Protection* 13:80–123
- Vaughn KC (1986) Immunocytochemistry of chloroplast antigens. In: Linskens HF, Jackson JF (eds) *Immunology in plant sciences*. Springer, Berlin, pp 247–258
- Vaughn KC (1987) Two immunological approaches to the detection of ribulose-1,5-bisphosphate carboxylase in guard cell chloroplasts. *Plant Physiol* 84:188–196
- Vaughn KC, Duke SO (1981a) Tissue localization of polyphenol oxidase in *Sorghum*. *Protoplasma* 108:319–327
- Vaughn KC, Duke SO (1981b) Histochemical localization of nitrate reductase. *Histochemistry* 72:191–198
- Vaughn KC, Duke SO (1984) Tentoxin stops the processing of polyphenol oxidase into an active protein. *Physiol Plant* 60:257–261
- Vaughn KC, Campbell WH (1988) Immunogold localization of nitrate reductase in maize leaves. *Plant Physiol* 88:1354–1357

- Vaughn KC, Vaughan MA (1988) Is ribulose biphosphate carboxylase present in guard cell chloroplasts? *Physiol Plantarum* 74:409–413
- Vaughn KC, Lax AR, Duke SO (1988) Polyphenol oxidase: the chloroplast oxidase with no established function. *Physiol Plant* 72:659–665
- Vaughn KC, Campbell EO, Hasegawa J, Owen HA, Renzaglia KS (1990) The pyrenoid is the site of ribulose 1,5-bisphosphate carboxylase/oxygenase accumulation in the hornwort (Bryophyta: Anthocerotae) chloroplast. *Protoplasma* 156:117–129
- Vaughn KC, Miller PD, Wilson KG (1981) Ultrastructural localization of polyphenoloxidase in *Aegopodium podagraria* L. *Cytobios* 31:27–36
- Vaughn KC, Vierling E, Duke SO, Alberte RS (1983) Immunocytochemical and cytochemical localizations of photosystem I and II. *Plant Physiol* 73:203–207
- Vesk M, Dwarte D, Fowler S, Hiller RG (1992) Freeze fracture immunocytochemistry of the light-harvesting pigment complexes in a cryptophyte. *Protoplasma* 170:166–176



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