Photosynthesis Research **73:** 265–272, 2002.

Minireview

Three decades in transport business: studies of metabolite transport in chloroplasts – a personal perspective

Hans-Walter Heldt
Abteilung Biochemie der Pflanze, Albrecht-von Haller-Institut fuer Pflanzenwissenschaften, Universität Göttingen, Untere Karspuele 2, 37073 Göttingen, Germany (e-mail: hheldt@gwdg.de; fax: +49-551-395749)

Received 4 July 2001; accepted in revised form 24 October 2001

Key words: ADP, ATP, bicarbonate, chloroplasts, dicarboxylates, Ingo Flügge, glucose, glucose-6-phosphate, Hans-Walter Heldt, metabolite translocators, phosphate, phosphoenolpyruvate, pyruvate, 3-phosphoglycerate, transport, triosephosphate, David Walker

Abstract

This article gives an historical overview of our group's research on various metabolite translocators of chloroplasts, such as the translocators for phosphorylated intermediates of the Calvin–Benson cycle and of glycolysis, of ADP and ATP, of dicarboxylates, of pyruvate and of hexoses; how it began and where it led to. Wherever appropriate, references will be made to research in other laboratories.

Abbreviations: 6P-P-translocator – glucose 6-phosphate–phosphate translocator; PEP-P-translocator – phosphoenolpyruvate-phosphate-translocator; triose P-P-translocator – triose phosphate-phosphate-translocator

Prelude: characterization of ATP/ADP transport across the inner membrane of mitochondria

I entered the field of plant biochemistry after working for about 10 years on animal metabolism. This experience proved to be a great asset. As a postdoc, I had been working on oxidative phosphorylation by animal mitochondria in the laboratory of Martin Klingenberg at the University of Marburg. There I studied the kinetics of the phosphorylation of endogenous and exogenous ADP in rat liver mitochondria, and the different effects of atractyloside on these processes. In the same laboratory, Erich Pfaff established the method of silicon-layer-filtering centrifugation for measuring the uptake of ADP and ATP into mitochondria. From our results, we arrived at the conclusion that, in their inner membranes, mitochondria contain a specific ATP/ADP translocator, facilitating the uptake of ADP and the export of ATP in the course of oxidative phosphorylation (Pfaff et al. 1965). At the beginning, this concept met with criticism, but soon afterwards it was fully accepted. Using silicon layer filtering centrifugation, I studied the properties of the mitochondrial ATP/ADP translocator in more detail and found that the translocator catalyzes a strict 1:1 counter exchange, and that the import of ADP and the export of ATP are driven by a membrane potential generated by mitochondrial electron transport (Heldt et al. 1972a).

What was known in 1968 about the permeability of the chloroplast envelope?

As an animal biochemist, I was rather ignorant of the metabolism involved in photosynthetic reactions, but I realized that chloroplasts share similarities with mitochondria. Being experienced in mitochondrial transport, I became curious as to whether chloroplasts also possess specific metabolite translocators. By then, it was known how to isolate, from leaves of spinach and pea, intact chloroplasts capable of performing CO_2assimilation (David Walker 1964; Elchanan Bamberger and Martin Gibbs 1965; Richard Jensen and

James Bassham 1966). In 1968, I attended the First International Congress on Photosynthesis in Freudenstadt, in the Black Forest, Germany, to learn what was known up to that time about metabolite transport in chloroplasts. At this meeting, the most valuable information for me was from David Walker's lecture on the permeability of the chloroplast envelope (Walker 1969). He showed impressive oxygen electrode traces of isolated chloroplasts performing CO_2 fixation in the presence of various metabolites, and from these results concluded convincingly that the intact envelope of chloroplasts is readily permeable to 3-phosphoglycerate and dihydroxyacetone phosphate, only little permeable to hexose phosphates, and virtually impermeable to the reduced form of nicotinamide adenine dinucleotide phosphate, NADPH and ferredoxin. I realized later that Bassham et al. (1968) had already deduced a permeability of the chloroplast for 3-phosphoglycerate from the distribution of labeled metabolites between intact chloroplasts and the suspension medium in the course of photosynthesis. Ulrich Heber et al. (1967) had arrived at a similar conclusion by using nonaqueous fractionation of frozen leaves to assay the subcellular distribution of labeled metabolites in whole leaves performing photosynthesis. Heber postulated from his results that dihydroxyacetone phosphate and 3-phosphoglycerate are transport metabolites of chloroplasts.

All of these studies indicated that the chloroplast envelope has a selective permeability, but it was not known whether specific translocators are responsible for this. We had the know-how to study metabolite transport into mitochondria by silicon layer filtering centrifugation. Thus, it was relatively easy for us to adapt this technique to chloroplasts. It turned out that this method worked even better with chloroplasts than with mitochondria, since chloroplasts, being larger, are centrifuged more rapidly through the silicon layer than the much smaller mitochondria are. At the Freudenstadt conference, I also learned how to prepare chloroplasts, when W. Cockburn, a collaborator of David Walker, told me the procedure for preparing intact chloroplasts. Thus, the First International Congress on Photosynthesis had a decisive influence on my subsequent work on plant metabolism. Three years later at the Second International Congress on Photosynthesis in Stresa (Italy), I was already in a position to report about the basic features of chloroplast metabolite transport (Heldt et al. 1972b). Since that time, I have attended all the International Congresses on Photosynthesis until the present.

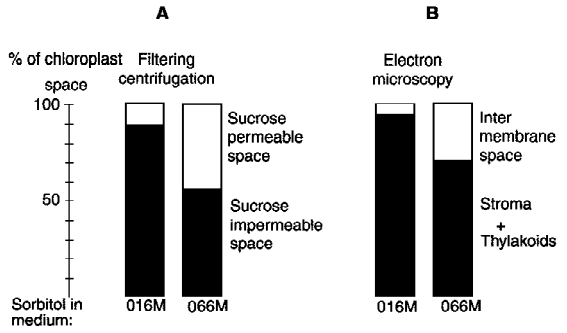

Figure 1. Comparison of the relative sizes of the sucrose permeable spaces of isolated spinach chloroplasts measured by filtering centrifugation with the sizes of the intermembrane spaces obtained by planimetry of electron micrographs [from Heldt et al. (1972b)].

Transport of ADP and ADP

Naturally, I first investigated whether chloroplasts possess an ATP/ADP translocator, and whether such would be similar to the mitochondrial translocator that I had been previously studying. It turned out that the chloroplast envelope has indeed an ATP/ADP translocator. Unlike the mitochondrial ATP/ADP translocator, however, it was not inhibited by atractyloside, and its nucleotide specificity was different from that of the mitochondrial translocator. For instance, it was completely inactive with GTP, CTP, and UTP. It became quite obvious that the chloroplast ATP/ADP translocator was not suited for the export of ATP generated by photophosphorylation, but rather for the supply of chloroplasts with ATP from the cytosol during the dark period (Heldt 1969). About 25 years later, the primary structure of the plastidic ATP/ADP translocator has been determined in the laboratory of Eckehard Neuhaus (Kampfenkel et al. 1995). From the primary structure, it can be deduced that the ATP/ADP translocator protein has a membrane topology with a 12 transmembrane helices motif, which is different from the 6+6 helix motif of mitochondrial translocators, but resembles that of prokaryotic and eukaryotic plasma membrane transporters.

Localization of chloroplastic metabolite translocators

As the chloroplast envelope consists of two membranes, the question arose, in which one is the ATP/ADP translocator localized. At different osmolarities of the suspension medium, we determined the permeability of intact chloroplasts to radioact-

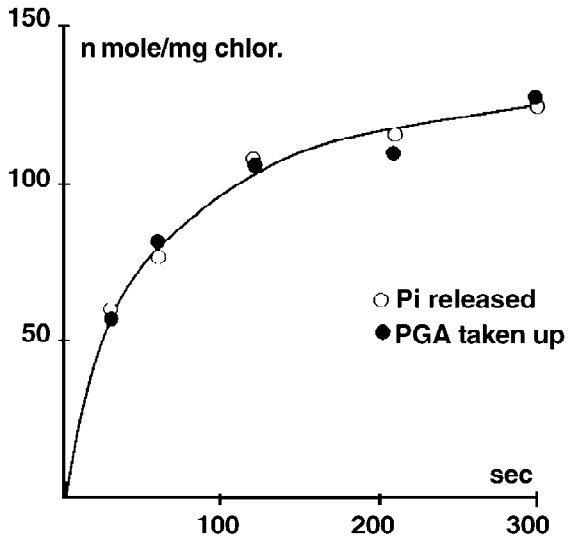

Figure 2. Simultaneous measurement of the uptake of ^{14}C-labeled 3-phosphoglycerate and the release of ^{32}P-phosphate by silicone layer filtering centrifugation. The spinach chloroplasts were pre-incubated with inorganic ^{32}P-phosphate in the dark; temperature 4 °C; darkness [from Fliege et al. (1978)].

ively labeled solutes, such as GTP, sucrose, dextran and H_2O. In parallel, we made electron micrographs of chloroplasts under these conditions (Figure 1). A comparison of these results revealed that the inner envelope membrane was the osmotic barrier of the chloroplasts and also the site of metabolite translocators, whereas the outer membrane appeared to be unspecifically permeable to metabolites of low molecular weight, e.g., nucleotides or sugar phosphates (Heldt and Frieder Sauer 1971). Later, it was shown that the permeability of the outer envelope membrane was due to a pore-forming protein, a putative porine (Ingo Flügge and Roland Benz 1984).

Transport of Calvin–Benson cycle intermediates across the chloroplast envelope

Next, my technician Lynn Rapley and I studied the transport of radioactively labeled phosphate and phosphorylated metabolites into spinach chloroplasts (Heldt and Rapley 1970). We observed a rapid uptake of phosphate and 3-phosphoglycerate, whereas the uptake of hexose- and pentose phosphates was low. The uptake of phosphate was competitively inhibited by triose phosphates and 3-phosphoglycerate, but not by 2-phosphoglycerate, and the uptake of 3-phosphoglycerate was inhibited by triose phosphates and phosphate. Moreover, the label was released from chloroplasts pre-incubated with radioactive phosphate upon the addition of phosphate, triose phosphate, and 3-phosphoglycerate. Likewise, the label was released from chloroplasts pre-incubated with ^{14}C-labeled 3-phosphoglycerate upon the addition of 3-phosphoglycerate, phosphate, and triosephosphates, but not of 2-phosphoglycerate or phosphoenolpyruvate. Further studies (Rainer Fliege et al. 1978) showed that this translocator catalyzes the transport of divalent anions in a strict 1:1 counter exchange (Figure 2). These and other findings led to the conclusion that, in their inner envelope membrane, chloroplasts contain a specific translocator, catalyzing a counter exchange of phosphate, 3-phosphoglycerate, and triose phosphate. From its very high activity, it was obvious that this translocator is a partial step in the overall process of CO_2 assimilation, as it facilitates the export from the chloroplasts of triose phosphates and 3-phosphoglycerate in exchange with inorganic phosphate. Therefore, initially we named this translocator *phosphate translocator*. As will be discussed later, several classes of plastidic phosphate translocators are now known. For this reason the phosphate translocator of C_3 plants described above is now more specifically termed *triose phosphate–phosphate-translocator*, in short triose P–P-translocator.

The transport was found to be inhibited by pyridoxal 5-phosphate and trinitrobenzene sulfonate, both reacting with lysine residues, phenylglyoxal which reacts with arginine residues, and β-chloromercuriphenyl sulfonate reacting with sulfhydryl groups (Fliege et al. 1978). It was also shown that the binding site of the triose P–P-translocator contains a lysine and an arginine residue, providing the two cationic charges for the binding of the doubly charged substrate anions, and that an SH-group, although not located at the substrate binding site, is essential for the translocator function (Flügge and Heldt 1979). These inhibitors turned out to be valuable tools for identifying the translocator protein. Incubation with trinitrobenzene sulfonic acid, followed by treatment with tritium-labeled sodium borohydride, showed the protein of the triose P–P-translocator to be a polypeptide with an apparent molecular weight of 29 000, as obtained by SDS-polyacrylamide gel electrophoresis. Moreover, the translocator protein was reconstituted functionally into liposomes (Flügge and Heldt 1981).

Subsequently, Ingo Flügge and his group succeeded in isolating the triose P–P-translocator from

spinach chloroplasts. From the resulting tryptic peptides, a full length cDNA clone was obtained and sequenced. In this way, the primary structure of a chloroplast metabolite translocator was elucidated for the first time (Flügge et al. 1989). By now a number of cDNAs for triose P–P-translocators from various plants have been sequenced, and all these sequences turned out to be highly homologous to each other (Flügge 1999).

Several observations indicated that plastidic phosphate translocators exist with a substrate specificity different from that of the triose P–P-translocator dealt with thus far. Maize mesophyll chloroplasts were found to transport C_3 compounds with the phosphate group attached to the C2-atom, e.g. phosphoenolpyruvate and 2-phosphoglycerate in exchange with phosphate (Steve Huber and Gerald Edwards 1977a; David Day and Hal Hatch 1981a; Armin Gross et al. 1990). In Flügge's group, a plastidic phosphate translocator protein from maize endosperm membranes was purified, and the corresponding cDNA cloned and sequenced, of which the resulting membrane protein, after reconstitution into liposomes, mediated the transport of inorganic phosphate in exchange with C_3 compounds phosphorylated in the C2 position, in particular phosphoenolpyruvate, but transported triosephosphate only poorly. It was, therefore, named phosphoenolpyruvate–phosphate-translocator, in short PEP–P–translocator (Karsten Fischer et al. 1997). It turned out that this plastidic translocator is present in both photosynthetic active tissues and in nongreen tissues. Apparently, the PEP–P-translocator occurs in plastids to various extents besides the triose P–P-translocator. One general function of the PEP–P-translocator is to facilitate the import of phosphoenol pyruvate into the plastids required for the synthesis of aromatic amino acids via the shikimate pathway.

The triose P–P-translocator as well as the PEP–P-translocator do not transport glucose 6-phosphate. In pea root plastids, however, we found that glucose 6-phosphate is transported in exchange with phosphate or triosephosphate (Sieglinde Borchert et al. 1989). Flügge's group has isolated this translocator and sequenced the corresponding cDNA (Birgit Kammerer et al. 1998). The glucose 6 P–P-translocator was found to be present mainly in plastids of heterotrophic tissues, e.g., maize root endosperm and potato tubers. This translocator mediates the uptake of glucose 6-phosphate into plastids for the synthesis of starch or as substrate of the oxidative pentose phosphate pathway.

It appears now that there is a family of plastidic phosphate translocators consisting of the triose P–P-translocators, the PEP–P-translocators, and the glucose 6 P–P-translocators. These three classes have different profiles in organ expression. In each class, the translocator proteins from various plants are highly homologous (75–95% identity), whereas the homology between the different classes is only about 35%. All members of the different classes are nuclearly encoded. The functional translocators are dimers, where each monomer consists of about 330 amino acids. From hydropathic analysis each monomer is predicted to consist of 5–7 transmembrane helices. Apparently, the plastidic phosphate translocators belong to a group of transporters with a 6+6 helix motif, as in the case of mitochondrial carrier proteins (Flügge 1999).

Uptake of inorganic carbon into chloroplasts

As it was not known then how inorganic carbon enters the chloroplast, Karl Werdan measured the uptake of radioactively labeled bicarbonate into isolated chloroplasts by means of silicon layer filtering centrifugation. The uptake turned out to be so rapid that it could not be resolved kinetically. Diamox, an inhibitor of carbonic anhydrase, decreased the rate of uptake, but the finally reached concentration was not affected. It turned out that the concentration of bicarbonate attained in the chloroplast stroma was a function of the bicarbonate concentration in the medium and of the pH gradient between the medium and the chloroplast stroma. These results clearly indicated that the accumulation of bicarbonate in the chloroplast stroma was due to a diffusion of CO_2 across the chloroplast envelope into the stroma, where it equilibrated with bicarbonate as catalyzed by carbonic anhydrase (Werdan and Heldt 1972). These results contradicted the notion discussed at that time that chloroplasts may have a bicarbonate translocator.

Transport of dicarboxylates

In our early experiments about metabolite transport into isolated chloroplasts, we also investigated the uptake of carboxylates (Heldt and Rapley 1970). These studies revealed that certain dicarboxylates, such as L-malate, oxaloacetate, 2-oxoglutarate, fumarate, succinate aspartate and glutamate, but not malonate, maleinate, citrate or monocarboxylates, are trans-

ported into the chloroplasts by specific counter exchange. Subsequently, a transport of dicarboxylates was also observed in chloroplasts from pea (Proudlove and Thurmann 1981) and maize mesophyll (Day and Hatch 1981b). Our detailed studies revealed that the transport of dicarboxylates into chloroplasts is facilitated by different translocators of overlapping specificity (Karl Lehner and Heldt 1978). Investigations of the effect of dicarboxylates on the uptake of 2-oxoglutarate suggested that the binding of 2-oxoglutarate occurred at a binding site different from that of other transported dicarboxylates (Ian Dry and Joe Wiskich 1983; Proudlove et al. 1984; K C Woo et al. 1984). An uptake of 2-oxoglutarate into and a release of glutamate from C_3 chloroplasts are required for NH_3assimilation and the photorespiratory NH_3 reassimilation via the glutamine synthetase/glutamate synthase pathway. K.C. Woo from Australia, who worked as a Humboldt research fellow in our laboratory, carried out a detailed study of the kinetics of the exchange of 2-oxoglutarate and glutamate in spinach chloroplasts. He demonstrated that two transport processes are involved, both catalyzing an exchange with malate: an uptake of 2-oxoglutarate in exchange with stromal malate via a 2-oxoglutarate translocator (which does not transport glutamate) and a release of glutamate in exchange with external malate via a dicarboxylate translocator. These processes result in a net 2-oxoglutarate/glutamate exchange with no net malate transport (Woo et al. 1987; Flügge et al. 1988). In addition to this, a translocator catalyzing the exchange of glutamine against glutamate has been found (Jianwei Yu and Woo 1988).

In Flügge's research group, the chloroplast 2-oxoglutarate translocator has been purified, and via corresponding peptides its full-length cDNA obtained and sequenced. The corresponding translocator protein consists of 569 amino acids and probably functions as a monomer (Andreas Weber et al. 1995). The chloroplastic 2-oxoglutarate translocator protein has a 12-helix motif, and thus is entirely different from the mitochondrial dicarboxylate translocators.

As mentioned before, our early experiments had shown that oxaloacetate is also transported in exchange with other dicarboxylates (Heldt and Rapley 1970; Lehner and Heldt 1978). The question arose whether chloroplastic oxaloacetate transport is suited to mediate a transfer of redox equivalents from the chloroplasts to the cytosol via a malate-oxaloacetate shuttle. Due to the malate dehydrogenase equilibrium (K_{equil} at pH 7 = 3×10^5), the cytosolic concentration of malate is expected to be orders of magnitude higher than that of oxaloacetate. During a stay in our laboratory, Hal Hatch from Canberra measured the uptake of ^{14}C-labeled oxaloacetate into chloroplasts from maize mesophyll cells and spinach leaves. The results clearly demonstrated that these chloroplasts contained a highly specific oxaloacetate translocator, with very low competition by an even 1000-fold excess of other dicarboxylates (Hatch et al. 1984). Subsequent studies with whole leaves, employing nonaqueous subcellular fractionation, gave evidence that a transfer of redox equivalents from the chloroplasts to the cytosol via a malate-oxaloacetate shuttle does indeed occur (Dieter Heineke et al. 1991).

This shuttle turned out to be part of a network. In C_3 plants it plays an important role in the photorespiratory pathway in supplying the peroxisomes with redox equivalents as required for the reduction of pyruvate. When looking more deeply into this problem, we found that the mitochondria can also be a source of such redox equivalents (Agepati Raghavendra et al. 1998). In contrast to animal mitochondria, which normally do not transport oxaloacetate, plant mitochondria turned out to have a specific oxaloacetate translocator facilitating a malate-oxaloacetate shuttle (Iris Hanning et al. 1999). Our results indicated that at ambient CO_2, the contribution of mitochondria and chloroplasts to the demand of redox equivalents in the peroxisomes is about 50:50 (Hanning and Heldt 1993). The permeability of the peroxisomes, which contain only one boundary membrane, had long been an enigma. We found that instead of translocators, peroxisomes have porin-like channels in their membrane, enabling the passage of malate and oxaloacetate (Sigrun Reumann et al. 1998).

Transport of pyruvate

Pyruvate is not transported by spinach chloroplasts, but is taken up readily by chloroplasts from mesophyll cells of C_4 plants, as shown first by Huber and Edwards (1977b). In C_4 metabolism, pyruvate arising from decarboxylation reactions in the bundle sheath cells is transported into the mesophyll chloroplasts in order to be converted there to phosphoenolpyruvate, which is the primary acceptor of CO_2. For theoretical reasons, it was to be expected that the transport of pyruvate into mesophyll chloroplasts proceeds against a concentration gradient. When we checked this, we found that pyruvate uptake in maize mesophyll chloroplasts proceeded indeed by active transport, which was light dependent and uncoupler sensitive (Flügge et al.

Figure 3. Participants of a 1968 Research Conference on CO_2 Fixation of Green Plants. Names of only some of the participants are listed here. Gerry Edwards and William Ogren are second and third from the left in the first row beside Raymond Chollet (in horizontal stripped shirt). Ulrich Heber and James Bassham are third and fourth from the left in the second row. Hans Heldt is third from the left in the third row; in the same row, Richard Jensen is behind James Bassham. Hal Hatch (in vertical striped shirt) is in the middle of the fourth row beside Raymond Chollet (in horizontal striped shirt). Ingo Flügge is the second from the right in the fifth row. Bob Buchanan is the fifth from the right in the last row.

1985). Apparently, pyruvate uptake was driven by a light-dependent pH gradient. Subsequently, Jun-ichi Ohnishi and Ruzei Kanai (1987a) also found light dependent pyruvate uptake into mesophyll chloroplasts from the C_4 plant *Panicum miliaceum*, but their results also indicated that a Na^+ gradient might be driving force for an active pyruvate uptake (Ohnishi and Kanai 1987b). To clarify the matter Jun-ichi Ohnishi came as a Humboldt research fellow to work in our group. When comparing pyruvate uptake into mesophyll chloroplasts from various C_4 plants, he found in species of the C_4-NAD^+-malic enzyme-type and C_4-PEP-carboxykinase-type species that a Na^+ gradient acted as driving force, whereas in the C_4-$NADP^+$-malic enzyme species maize, the driving force is a H^+ gradient (Ohnishi et al. 1990). It appears form these findings that, depending on the species, active pyruvate transport into C_4 mesophyll chloroplasts is driven either by an H^+ or a Na^+gradient.

Hexose transport

Together with Ulrich Heber, we investigated the transport of monosaccharides into spinach chloroplasts. We found that several hexoses and pentoses, such as D-glucose, D-mannose, D-fructose, D-xylose and D-ribose, but essentially no L-glucose, are transported into chloroplasts with a saturation characteristic (Gisela Schaefer et al. 1977). The approximate K_m for D-glucose was found to be 20 mm. The specificity of this translocator-mediated hexose transport turned out to be strikingly similar to the specificity of the glucose carrier in human erythrocytes (Le Fevre 1961) and

liver cells (Hartmut Baur and Heldt 1977). Recently, Ingo Flügge and his group identified the protein of the glucose translocator as a component of the inner envelope membrane. From it, peptides were derived and sequenced, and the latter used to isolate from spinach and various other plants cDNA clones encoding a putative plastidic glucose translocator (Andreas Weber et al. 2000). It appears from these results that the plastidic glucose translocator contains 12 transmembrane helices and is closely related to the mammalian glucose translocators of the GLUT (human erythrocyte glucose transporter) family, but is different from all other plant hexose translocators that have been characterized to date.

The main function of this chloroplastic glucose translocator appears to be the export of glucose deriving as a product of the degradation of starch in the chloroplasts. Our early experiments with isolated spinach chloroplasts have shown that the degradation of chloroplastic starch proceeds about half by phosphorylysis and about half by hydrolysis, the latter resulting in the release of glucose (Mark Stitt and Heldt 1981). In the meantime, there have been more indications that glucose is formed to large extent during the mobilization of chloroplast starch (for reference see Weber et al. 2000).

To acquaint the readers with the people involved in research in the area of translocation of metabolites and related areas, I present here a 1968 group photograph (see Figure 3). James Bassham, Gerald Edwards, Ingo Flügge, Hal Hatch, Ulrich Heber, Hans Heldt, and Richard Jensen, mentioned in this article, can be seen in this photograph. Among others, John Bennett, Bob Buchanan, Ray Chollet, Doug Jordon, George Lorimer, Archie Portis, Mike Salvucci and Chris Somerville, shown here, are mentioned in other articles in this special issue. It may be a good exercise for the readers to match names with photos!

Outlook

In recent years, our studies of the properties of chloroplast translocators have been supplemented by cloning and molecular characterization of corresponding translocator proteins. But the job is not finished yet. Several chloroplastic translocators, including the translocators for malate and glutamate, oxaloacetate, glutamine, and pyruvate, remain to be characterized at the molecular level.

Acknowledgment

I am grateful to the Deutsche Forschungsgemeinschaft for its constant and efficient funding of the research summarized in this historical Minireview.

References

Bamberger ES and Gibbs M (1965) Effect of phosphorylated compounds and inhibitors on CO_2 fixation by intact spinach chloroplasts. Plant Physiol 40: 919–926

Bassham JA, Kirk M and Jensen RG (1968) Photosynthesis by isolated chloroplasts. Biochim Biophys Acta 153: 211–218

Baur H and Heldt HW (1977) Transport of hexoses across the liver-cell membrane. Eur J Biochem 74: 397–403

Borchert S, Grosse H and Heldt HW (1989) Specific transport of phosphate, glucose 6-phosphate, dihydroxyacetone phosphate and 3-phosphoglycerate into amyloplasts from pea roots. FEBS Lett 253: 183–186

Day DA and Hatch MD (1981a) Transport of 3-phosphoglyceric acid, phosphoenolpyruvate, and inorganic phosphate in maize mesophyll chloroplasts, and the effect of 3-phosphoglyceric acid on malate and phosphoenolpyruvate production. Arch Biochem Biophys 211: 743–749

Day DA and Hatch MD (1981b) Dicarboxylate transport in maize mesophyll chloroplasts. Arch Biochem Biophys 211: 738–742

Dry IB and Wiskich JT (1983) Characterization of dicarboxylate stimulation of ammonia, glutamine and 2-oxoglutarate-dependent O_2evolution in isolated pea chloroplasts. Plant Physiol 72: 291–296

Fischer K, Kammerer B, Gutensohn M, Arbinger B, Weber A, Haeusler RE and Flügge UI (1997) A new class of plastidic translocators: a putative link between primary and secondary metabolism by the phosphoenolpyruvate/phosphate antiporter. Plant Cell 9: 453–462

Fliege R, Flügge UI, Werdan K and Heldt HW (1978) Specific transport of inorganic phosphate, 3-phosphoglycerate and triose phosphates across the inner membrane of the envelope in spinach chloroplasts. Biochim Biophys Acta 502: 232–247

Flügge UI (1999) Phosphate translocators in plastids. Annu Rev Plant Physiol Plant Mol Biol 50: 27–45

Flügge UI and Benz R (1984) Pore-forming activity in the outer membrane of the chloroplast envelope. FEBS Lett 169: 85–89

Flügge UI and Heldt HW (1979) Phosphate translocator in chloroplasts: identification of the functional protein and characterization of its binding site. In: Quagliariello E, Palmieri F, Papa S and Klingenberg EM (eds) Function and Molecular Aspects of Biomembrane Transport, pp 373–382. Elsevier/North Holland Biomedical Press, Amsterdam

Flügge UI and Heldt HW (1981) The phosphate translocator of the chloroplast envelope. Isolation of the carier protein and reconstitution of transport. Biochim Biophys Acta 638: 296–304

Flügge UI, Stitt M and Heldt HW (1985) Light driven uptake of pyruvate into mesophyll chloroplasts from maize. FEBS Lett 183: 335–339

Flügge UI, Woo KC and Heldt HW (1988) Characteristics of 2-oxoglutarate and glutamate transport in spinach chloroplasts. Planta 174: 534–541

Flügge UI, Fischer K, Gross A, Sebald W, Lottspeich F and Eckerskorn C (1989) The triose phosphate-3-phosphoglycerate-phosphate translocator from spinach chloroplasts: nucleotide sequence of a full-length cDNA clone and import of the *in*

vitro synthesized precursor protein into chloroplasts. EMBO J 8: 39–46

Gross A, Brueckner G, Heldt HW and Flügge UI (1990) Comparison of the kinetic properties, inhibition and labelling of the phosphate translocator from maize and spinach mesophyll chloroplasts. Planta 180: 262–271

Hanning I and Heldt HW (1993) On the function of mitochondrial metabolism during photosynthesis in spinach leaves. Partitioning between respiration and export of redox equivalents and precursors for nitrate assimilation products. Plant Physiol 103: 1147–1154

Hanning I, Baumgarten K, Schott K and Heldt HW (1999) Oxaloacetate transport into plant mitochondria. Plant Physiol 119: 1025–1031

Hatch MD, Droescher L and Heldt HW (1984) A specific translocator for oxaloacetate transport in chloroplasts. FEBS Lett 178: 15–19

Heber U, Santarius KA, Hudson MA and Hallier UW (1967) Intrazellulaerer Transport von Zwischenprodukten der Photosynthese im Photosynthese-Gleichgewicht und im Dunkel-Licht-Dunkel-Wechsel. Z Naturforsch 22b: 1189–1199

Heineke D, Riens B, Grosse H, Hoferichter P, Peter U, Flügge UI and Heldt HW (1991) Redox transfer across the inner chloroplast envelope membrane. Plant Physiol 95: 1131–1137

Heldt HW (1969) Adenine nucleotide translocation in spinach chloroplasts. FEBS Lett 5: 11–14

Heldt HW and Rapley L (1970) Specific transport of inorganic phosphate, 3-phosphoglycerate and dihydroxyacetone phosphate and of dicarboxylates across the inner membrane of spinach chloroplasts. FEBS Lett 10: 143–148

Heldt HW and Sauer F (1971) The inner membrane of the chloroplast envelope as the site of specific metabolite transport. Biochim Biophys Acta 234: 83–91

Heldt HW, Klingenberg M and Milovancev M (1972a) Differences between the ATP/ADP ratios in the mitochondrial matrix and in the extramitochondrial space. Eur J Biochem 30: 434–440

Heldt HW, Sauer F and Rapley L (1972b) Differentiation of the permeability properties of the two membranes of the chloroplast envelope. In: Proceedings of the Second International Congress on Photosynthesis, pp 1345–1355. Dr W. Junk Publishers, The Hague

Huber SC and Edwards GE (1977a) Transport in C_4 mesophyll chloroplasts. Evidence for an exchange of inorganic phosphate and phosphoenolpyruvate. Biochim Biophys Acta 462: 603–612

Huber SC and Edwards GE (1977b) Transport in C_4 mesophyll chloroplasts. Characterization of the pyruvate carrier. Biochim Biophys Acta 462: 583–602

Jensen RG and Bassham JA (1966) Photosynthesis by isolated chloroplasts. Proc Natl Acad Sci USA 56: 1095–1101

Kammerer B, Fischer K, Hilpert B, Schubert S, Gutensohn M, Weber A and Flügge UI (1998) Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: the glucose 6-phosphate/phosphate antiporter. Plant Cell 10: 105–117

Kampfenkel K, Moehlmann T, Batz O, van Montagu M, Inze D and Neuhaus HE (1995) Molecular characterization of an *Arabidopsis thaliana* cDNA encoding a novel putative adenylate translocator of higher plants. FEBS Lett 74: 351–355

Le Fevre PG (1961) Sugar transport in the red blood cell: structure, activity, relationship in substrates and antagonists. Pharmacol Rev 13: 39–70

Lehner K and Heldt HW (1978) Dicarboxylate transport across the inner membrane of the chloroplast envelope. Biochim Biophys Acta 501: 531–544

Ohnishi J and Kanai R (1987a) Light dependent uptakeof pyruvate by mesophyll chloroplasts of a C_4 plant *Panicum miliaceum.* Plant Cell Physiol 28: 243–251

Ohnishi J and Kanai R (1987b) Na^+ induced uptake of pyruvate into mesophyll cells of a C_4 plant *Panicum miliaceum*. FEBS Lett 219: 347–350

Ohnishi J, Flügge UI, Heldt HW and Kanai R (1990) Involvement of Na^+ in active uptake of pyruvate in mesophyll chloroplasts of some C_4 plants. Plant Physiol 94: 950–959

Pfaff E, Klingenberg M and Heldt HW (1965) Unspecific permeation and specific exchange of adenine nucleotides in liver mitochondria. Biochim Biophys Acta 104: 312–315

Proudlove MO and Thurman DA (1981) The uptake of 2-oxoglutarate and pyruvate by isolated pea chloroplasts. New Phytol 88: 255–264

Proudlove MO, Thurman DA and Salisbury J (1984) Kinetic studies on the transport of 2-oxoglutarate and L-malate in isolated pea chloroplasts. New Phytol 96: 1–8

Raghavendra AS, Reumann S and Heldt HW (1998) Participation of mitochondrial metabolism in photorespiration. Reconstituted system of peroxisomes and mitochondria from spinach leaves Plant Physiol 116: 1333–1337

Reumann S, Maier E, Heldt HW and Benz R (1998) Permeability properties of the porin of spinach leaf perosixomes. Eur J Biochem 251: 359–366

Schaefer G, Heber U and Heldt HW (1977) Glucose transport into spinach chloroplasts. Plant Physiol 60: 286–289

Stitt M and Heldt HW (1981) Physiological rates of starch breakdown in isolated intact spinach chloroplasts. Plant Physiol 68: 755–761

Walker DA (1964) Improved rate of carbon dioxide fixation by illuminated chloroplasts. Biochem J 92: 22c–23c

Walker DA (1969) Permeability of the chloroplast envelope. Proceedings of the First International Congress on Photosynthesis. Progress in Photosynthesis Research Vol 1: 250–257

Weber A, Menzlaff E, Arbinger B, Gutensohn M, Eckerskorn C and Flügge UI (1995) The 2-oxoglutarate/malate translocator of chloroplast envelope membranes: molecular cloning of a transporter containing a 12-helix motif and expression of the functional protein in yeast cells. Biochemistry 34: 2621–2627

Weber A, Servaites JC, Geiger DR, Kofler H, Hille D, Groner F, Hebbeker U and Flügge UI (2000) Identification, purification and molecular cloning of a putative plastidic glucose translocator. Plant Cell 12: 787–801

Werdan K and Heldt HW (1972) Accumulation of bicarbonate in intact chloroplasts following a pH gradient. Biochim Biophys Acta 283: 430–441

Woo KC, Flügge UI and Heldt HW (1984) Regulation of 2-oxoglutarate and dicarboxylate transport in spinach chloroplasts by ammonia in the light. In: Sybesma V (ed) Proceedings of the Sixth International Congress on Photosynthesis, Vol 3, pp 685–688. Dr W Junk Publishers, The Hague, The Netherlands

Woo KC, Flügge UI and Heldt HW (1987) A two translocator model for the transport of 2-oxoglutarate and glutamate during ammonia assimilation in the light. Plant Physiol 84: 624–632

Yu C and Woo KC (1988) Glutamine transport and the role of the glutamine translocator in chloroplasts. Plant Physiol 88: 1048–1054