Letters

The energetics of the chloroplast Tat protein transport pathway revisited

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Recently, a discussion session concerning protein trafficking in photosynthetic organisms was held at the 13th International Congress on Photosynthesis, which took place in Montreal during the week of 28 August 2004. Of particular interest was a controversy about the actual energy requirements for the so-called 'ΔpH-dependent' protein transport pathway of plant and algal thylakoid membranes. This letter is intended to communicate the outcome of this discussion and invite further commentary.

Transport of proteins into the thylakoid lumen occurs via either the chloroplast Sec pathway or the cpTat pathway. The cpTat pathway was first discovered in thylakoids and was long referred to by one of its distinguishing features, the ability to drive proteins into the lumen *in vitro* using just the chemiosmotic pH gradient across the thylakoid membrane [1,2]. Recently, Giovanni Finazzi *et al.* [3] challenged the requirement for a pH gradient, showing that transport of Tat pathway substrates can occur *in vivo* in the absence of a ΔpH.

In their experiments, these authors established conditions under which the lumen and stromal pH were the same, either in a *Chlamydomonas* mutant lacking subunits of the CF1 ATPase, or after application of nigericin to wild-type *Chlamydomonas* cells or barley leaves. Under these conditions, transport of several different Tat substrates was unperturbed. That is, in their hands, the ΔpH-dependent pathway did not depend on the ΔpH.

The 13th International Congress on Photosynthesis offered a venue for some of the researchers impacted by this new twist in chloroplast protein transport to meet for a formal discussion of the issues. A discussion session led by Steve Theg and Ken Cline, two researchers who have worked for some time on the cpTat pathway, was attended by two authors of the Finazzi *et al.* paper, Giovanni Finazzi and Francis-André Wollman. Our ensuing discussion went a long way towards resolving our respective differences and might provide insight into the role of the $\Delta\tilde{\mu}_{H^+}$ in transporting proteins in this pathway.

In their publication, Finazzi *et al.* considered two possibilities for the continued operation of the cpTat pathway in thylakoids without a ΔpH. First, the *in vivo* experiments might contain some factor(s) that is missing in the *in vitro* system that alters the energetic requirements of the transport reaction. This interpretation invokes an entropic role for the ΔpH, which is consistent with recent findings from the Cline laboratory [4] that assembly of the Tat translocase in isolated thylakoids is also dependent on the pH gradient. The assembly status of the Tat translocase *in vivo* or even in intact chloroplasts is not known.

The second possible explanation is that the transmembrane Δψ, as well as the ΔpH, can power the cpTat pathway. It was pointed out by Tom Avenson and Jeff Cruz from David Kramer's laboratory that previous experiments with isolated thylakoids were conducted under conditions that, although standard for the field, have recently been shown to reduce the steady-state Δψ substantially [5].

However, neither of the two hypotheses presently account for the previous reports that the strict *in vitro* requirement for ΔpH for thylakoid protein transport also holds in reactions run with intact chloroplasts, an experimental system that should closely resemble the thylakoid environment *in vivo*.

Should the $\Delta\tilde{\mu}_{H^+}$ hypothesis prove true, it would signal an important insight into the process. The equivalency of the Δψ and ΔpH suggests that, similar to the ATP synthase, the cpTat pathway operates via a proton well [6]; one consequence of that would be a tight coupling of proton flow to protein transport. Consistent with this idea is a recent finding of Nathan Alder and Steve Theg [7] in which the ratio of protons to proteins transported was determined to be constant under different experimental conditions for one particular substrate. Such constancy is not expected if H^+ ions are required to activate the cpTat channel via protonation of some amino acid residue (compare with Ref. [8]). A brief discussion on how to determine experimentally if the Δψ and ΔpH are equivalent, or if certain *in vitro* steps require ΔpH, did not yield clear cut answers, but at least we are now all in agreement about the nature of the question.

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No need to shift the paradigm on the metabolic pathway to transitory starch in leaves

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Starch is a major storage compound in higher plants and is present at high levels in leaves and storage organs. The classical pathway leading to starch biosynthesis includes synthesis of ADP-glucose (ADPG), the nucleotide sugar precursor required for elongation of an existing starch core primer, in the plastidic stroma [1]. ADPG synthesis is catalysed by ADPG-pyrophosphorylase (AGPase), which is known to be a highly regulated enzyme in all plants analysed to date (e.g. spinach, potato, pea, *Arabidopsis*, rice, wheat and barley) [2]. Moreover, it has recently been shown that AGPase from potato, *Arabidopsis* and pea leaves undergoes post-translational redox-modification and is activated in the light during ongoing starch biosynthesis in the stroma [3]. By contrast, starch biosynthesis in endosperm cells of cereals such as maize and barley starts with the cytosolic synthesis of ADPG followed by the subsequent import of this compound into the storage plastid [4]. However, this synthesis is dependent on an extraplastidial AGPase [4], and Brittle1, a transport protein, is believed to be responsible for importing ADPG [5,6].

In contrast to all previous publications on starch synthesis in non-cereals, Javier Pozueta-Romero and colleagues recently published a paper concluding that starch biosynthesis in potato leaves starts with the synthesis of ADPG in the cytosol [7]. The authors believe that this synthesis is catalysed by a bypass reaction of the cytosolic enzyme sucrose synthase, known as a sucrose mobilizing enzyme, which catalyses the cleavage of this disaccharide into fructose and UDP-glucose (UDPG) [8–10]. By expressing the bacterial enzyme ADPG-pyrophosphatase (shown to be more or less specific for ADPG hydrolysis) in the potato cytosol the authors demonstrated that the level of ADPG in leaves and the levels of starch in leaves and tubers are reduced [7]. The authors concluded from this observation and their own previous results [11] that ADPG in potato leaves is synthesized in the cytosol and enters the plastid via an as yet uncharacterized transporter [7].

Although the observation of Pozueta-Romero and colleagues [7] is interesting and deserves further experimental attention, we feel that the conclusions are not consistent with the vast amount of genetic and biochemical literature that argues against cytosolic ADPG production via sucrose hydrolysis in the mesophyll cells of potato plants.

The proposal of cytosolic ADPG production in non-cereal plants and the observation of reduced levels of starch in mutants expressing a cytosolically located ADPG hydrolysing enzyme contrasts with the following observations:

(i) *Arabidopsis* mutants lacking either plastidic phosphoglucose mutase (PGM) or plastidic AGPase lack starch in the leaf tissue [12–14]. If the majority of ADPG is synthesized in the cytosol, rather than in the stroma, these mutants should contain unaltered levels of starch. Although Pozueta-Romero and colleagues do mention starchless mutants, the biochemical consequences arising from their existence are ignored. In particular, the proposed role of AGPase in the retrieval of hexose units deriving from starch degradation during ongoing starch biosynthesis is not consistent with the starchfree phenotype of the *Arabidopsis* pPGM mutant, which contains a functional AGPase. If starch were formed by imported ADPG in the pPGM mutant, phosphorylytic starch breakdown would yield glucose 1-phosphate (Glc1P), the substrate of AGPase; given that the main product of amylolytic starch degradation is maltose [15], which is exported from the chloroplast via a specific transporter [16], unphosphorylated glucose would only represent a minor portion of the starch degradation products. Moreover, in *Arabidopsis*, a lack of chloroplastic glucan phosphorylase has no impact on diurnal changes in starch content [17], whereas the

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