Postdoc Fellowships for non-EU researchers

Mid-term Report

Name	Iftekhar Alam
Selection	
Host institution	UCL
Supervisor	Marc Boutry
Period covered by this report	from 1/08/2015 to 31/1/2016
Title	Regulation and function of plant plasma membrane H^+-ATPase

1. Objectives

Plant growth and development depend on the ability of plant cells to acquire minerals and nutrients from the external medium. Any failure in this process seriously handicaps the plant fitness and, in the worst case, can lead to plant death. The plasma membrane H^+-ATPase plays a major role in this process by establishing an electrochemical proton gradient across the plasma membrane. This, in turn, activates a whole range of secondary transporters that move ions and nutrients into and out of the cell, sometimes against huge concentration gradients. In addition, the H^+-ATPase has been proposed to play an important role in cell elongation by acidifying the apoplasm and so activating enzymes, such as expansins, involved in cell elongation (acid growth theory[1]). However, this theory is still controversial[2].

H^+-ATPase is a highly regulated enzyme. In particular, it has a C-terminal auto-inhibitory domain that can be deactivated by phosphorylation of the penultimate residue, a Thr, and the subsequent binding of regulatory 14-3-3 proteins[3].

To better understand the roles of the H^+-ATPase in transport activation and cell elongation, the host laboratory has generated a mutant of the H^+-ATPase isoform 4 (PMA4), the most highly expressed isoform, lacking the C-terminal auto-inhibitory domain. This truncated isoform (ΔC-PMA4) is therefore constitutively activated. To better understand the role of H^+-ATPase in cell elongation, ΔC-PMA4 was expressed in *Nicotiana tabacum* BY-2 cells under the control of a heat-inducible transcription promoter. Transferring cells from 25 to 37°C for 2 h induced ΔC-PMA4 expression and resulted in a 33 to 107 % increase of cell size according to the transgenic line (Niczyj et al., in preparation). The objective of this project consists of understanding how the expression of an activated H^+-ATPase results in cell expansion. We sought to address this question using a proteomic approach. Plasma membranes have to be prepared from wild-type and ΔC-PMA4-expressing cells and their protein content will be then compared by mass spectrometry analysis.

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[1] Hager A (2003) Role of the plasma membrane H^+-ATPase in auxin-induced elongation growth: historical and new aspects. J Plant Res 116: 483-505

[2] Kutschera U (2006) Acid growth and plant development. Science 311: 952-953

[3] Duby G, Boutry M (2009) The plant plasma membrane proton pump ATPase: a highly regulated P-type ATPase with multiple physiological roles. Pflugers Arch 457: 645-655

Palmgren MG (2001) Plant plasma membrane H^+-ATPases: powerhouses for nutrient uptake. Annu Rev Plant Physiol Plant Mol Biol 52: 817-845

2. Preliminary results

Our starting material consisted of two wild-type cell lines as well as four transgenic lines expressing ΔC-PMA4 under the control of the heat shock promoter. Cell cultures at exponential phase were shifted from 25 to 37°C for 2 h and then put back at 25°C for 10 h. Cells were harvested, grinded and a microsomal fraction was prepared by differential centrifugation. A plasma membrane-enriched fraction was then obtained by phase partition. SDS-PAGE and western blotting analysis using an anti-H^+-ATPase antibody indicated that a 5 to 6-fold enrichment of this plasma membrane marker in the wild-type samples. Since ΔC-PMA4 is about 10 kDa smaller than the WT H^+-ATPase, it could be easily identified by gel electrophoresis. Indeed the ΔC-PMA4-expressing cells clearly showed the presence of the truncated H^+-ATPase form in addition to the endogenous full size form. Since the ΔC-PMA4 was provided with a 6-His tag, we could confirm its presence by western blotting using an anti-His antibody. Enrichment of the plasma membrane fraction was confirmed by the increased ATPase activity measured in the control wild-type samples. In addition, comparison of the wild-type and ΔC-PMA4-expressing samples demonstrated the higher ATPase and proton pumping activity of the latter.

The proteomic approach was then carried out. Two-dimensional gel electrophoresis is not appropriate for membrane proteins. We therefore used a gel-free proteomic approach. Triplicate of plasma membrane proteins (100 µg) from the two wild-type and the four ΔC-PMA4-expressing samples were digested with trypsin and then labeled with Isobaric tags for relative and absolute quantitation (iTRAQ). The peptides were collected and separated by two-dimensional HPLC chromatography combining ionic exchange and reverse phase. About 1200 fractions were analyzed by mass spectrometry (MALDI-TOF/TOF). The mass data were then compared to data bases (NCBI) so as to identify proteins.

More than 400 proteins were identified with a good score ($p > 0.95\%$). We then identified those proteins that were more (> 2-fold) or less (< 0.5-fold) expressed in the ΔC-PMA4-expressing samples compared to the wild-type samples. Thirteen proteins displayed higher expression while seventeen proteins showed reduced expression upon ΔC-PMA4 expression. Some of these proteins are now being analyzed in more detail. Among these, one was identified as an unnamed protein product from *N. tabacum* (gi/257711663), which, after database search (blast), turned out to be homologous to a harpin inducing protein. This protein belongs to a family known to be involved in the hypersensitive response to virus infection as well as in leaf and flower senescence. Another protein corresponds to a receptor kinase (gi/697149354). This protein has transmembrane domains and is predicted to be a kinase. We can hypothesize that this protein might be involved in the regulation of membrane transporters. Finally, let us point to the DREPP2 protein (gi/8017996), a member of the developmentally regulated plasma membrane polypeptides.

3. Perspectives

There are two possible future directions of our project. One consists of analyzing in more detail the role of the plasma membrane proteins, the concentration of which increases or decreases after expression of ΔC-PMA4. We are particularly interested in the kinases which might be involved in the regulation of the H^+-ATPase.

The other possible perspective consists of expressing ΔC-PMA4 in plants. We know that constitutive expression in the whole plant is toxic and counter-selected. We thus think of expressing ΔC-PMA4 in particular cell types, such as root hairs. These are involved in mineral nutrition from the soil. Expressing ΔC-PMA4 might result in a higher proton electrochemical gradient across the plasma membrane and thus activated transport of minerals. This expression might also result in cell enlargement as shown for culture cells. In this case, root hair extension might also be profitable to improved nutrient uptake. For this

specific expression, we have access to root hair-specific transcription promoters obtained from our IUAP partner in Gent (Lieven De Veylder).

4. Valorisation/Diffusion

A manuscript reporting on the proteomic analysis of plasma membrane is in preparation.

These data will also be reported at international meetings.

5. Miscellaneous

This fellowship was interrupted (31 January 2016) following the decision of the home institution to repatriate Dr Alam for organizational reasons. It is hoped that Dr Alam will be allowed to return to the host laboratory at UCL in order to resume his fellowship. In the meantime, Dr Alam and Dr Boutry have agreed to go on with this project in the framework of a collaboration between both institutions.