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Betr. Comments to Milada Čovanová's thesis on "AUXIN BINDING PPROTEIN 1 (ABP1) and its role in the auxin management in plant cells"

As ABP1 expert Richard Napier once put it, "working on ABP1 is like walking in a minefield". This is especially true since the breakthrough discovery of a new class of auxin receptors in 2005. After that event, ABP1 was "out", and I don't refer to the extracellular localization of this receptor candidate here. Considering the impact of this discovery on the auxin community and the rapid shift of the interest to the new receptors, Mrs. Čovanová's difficult ABP1 project was carried out in difficult times for ABP1 researchers.

What was the situation at the beginning of her project? ABP1 had been around since the days of Hertel (1971) and Löbler and Klämbt (1985). After the 2005 discovery of TIR1 and the AFBs, people were aware of ABP1 as an additional receptor candidate, but the number of auxin effects clearly linked to this protein was very limited. Most of these responses like membrane hyperpolarization or protoplast swelling were difficult to interpret in the context of the classical auxin functions like elongation growth, cell differentiation, tropisms etc. The elucidation of the role of ABP1 was further complicated by the fact that ABP1 knockout plants are embryo-lethal.

Because of these problems ABP1 research was slowly progressing, while there were a number of breakthroughs in the in our molecular understanding of auxin transport and the generation of physiologically important auxin gradients. On the very date of Milada Čovanová's defence one of the key auxin transport researchers, Jiri Friml, will receive the 750000 € Körber prize in my home city.

Given this situation, it makes sense that Milada Čovanová attempted a broad range first screen for additional, better defined molecular functions of ABP1, and that she had a

special interest in possible links of ABP1 to the activity and distribution of auxin transporters. She established a system in BY2 cells in which ABP1 could be overexpressed, constitutively or induced. Alternatively ABP1 expression could be suppressed constitutively by the antisense technique. Co-expression of other genes like GFP-labeled auxin transporter genes was also possible. The most interesting and groundbreaking part is her discovery that ABP1 is linked to PIN cycling and, therefore, to auxin transport (pp37-46). She addressed the role of ABP1 on PIN-cycling in a very elegant system by monitoring the fluorescence recovery after photobleaching (FREP) in PIN1-GFP expressing cell lines (Fig. 5.14). ABP1 overexpression in this system slowed down the appearance of PIN1 at the plasma membrane, and this effect could be reversed by auxin treatment. The ABP1 effect was not influenced by Brefeldin A, an inhibitor of membrane flow, but was abolished by Tph23, an inhibitor of endocytosis. These results prove for the first time ABP1 having a role in controlling PIN-cycling, and doing so by modulating endocytosis, but not exocytosis. This is an important piece of new information that will pave the way for further studies. It is well possible that the membrane flow of other proteins may be influenced by ABP1 in the same way, which could be analyzed using the same technique.

More recently Milada Čovanová shifted her interest to auxin metabolism, where she found that that auxin conjugation was suppressed when ABP1 and the ER-localized auxin carrier PIN5 were overexpressed. It was long known that ABP1, due to its KDEL signal peptide, is predominantly localized at the lumen of ER. The finding that ABP1 and auxin in the ER influence IAA conjugation – which is known to take place in the ER – might be an important hint for a function of ER-ABP1

I mentioned before that Mrs. Čovanová did not characterize one single mechanism in depth, but screened for new possible functions for ABP1. With this approach there will be a number of negative findings, and most positive findings will require some followup research. This is clearly the case. However this piece of research, especially the FRAP data, gives an excellent starting point for a re-evaluation of the role of ABP1 and should be published as soon as possible in a high-impact journal. I am happy to see the dataset has been submitted for publication to Plant Cell, which is a top journal, with Milada Čovanová as a first author. By the way Milada Čovanová made it on the author's line of an earlier Science paper by contributing some small but important data related to this thesis work.

Generally, Mrs. Čovanová critically discusses her data in an appropriate way and refers to the relevant literature of her field. As demonstrated in this thesis and in discussions I had with her during conferences she has a good working knowledge of plant molecular biology, plant cell biology and physiology and plant signaling.

Making a promising, intriguing discovery shedding some light on the function of a key plant protein and hopefully getting it published in a good journal – what can you expect more from your PhD-time?

The quality of this thesis clearly qualifies for a Ph.D. degree, and I consider it appropriate for defense without restrictions.

H. Lüthen

Hamburg, August 19, 2010 (Hartwig Lüthen)

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Eva Zazamilova told me that it is also appreciated to explicitely fix some questions as a starting point for the discussion.

1) Can you speculate on possible modes of crosstalk between ABP1 and TIR1?

2) Directly to the thesis: The most interesting part of your work is clearly the establishment of a link between PIN-cycling and ABP1 by FRAP. Is this an auxin-specific response?

3) ABP1 has been called a receptor, a receptor candidate or even a "red herring". What is your position?

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