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Article Addendum

# Does Auxin Binding Protein 1 Control Both Cell Division and Cell Expansion?

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*The Auxin Binding Protein 1 is Essential for the Control of Cell Cycle*

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## ABSTRACT

The Auxin-Binding Protein 1 (ABP1) was identified over 30 years ago thanks to it's high affinity for active auxins. ABP1 plays an essential role in plant life yet to this day, its function remains 'enigmatic.' A recent study by our laboratory shows that ABP1 is critical for regulation of the cell cycle, acting both in $G_1$ and at the $G_2$/M transition. We showed that ABP1 is likely to mediate the permissive auxin signal for entry into the cell cycle. These data were obtained by studying a conditional functional knock-out of ABP1 generated by cellular immunization in the model tobacco cell line, Bright Yellow 2.

## UNEQUIVOCAL ROLE FOR ABP1 IN CELL DIVISION

ABP1 has been demonstrated to be the auxin receptor involved in very early auxin responses such as the modification of ion fluxes at the plasma membrane.[1-3] Results obtained with plants overexpressing *ABP1*[4] or *ABP1* knock-out lines[5] emphasized a role for this protein in auxin-mediated cell expansion. A role for ABP1 in cell division has also been evoked[6] and characterisation of the *Arabidopsis abp1* loss of function mutant supports a dual role of the protein in cell expansion and cell division during early embryogenesis. In the referenced work, we've shown by immunomodulation of ABP1 that this auxin receptor acts on the control of cell cycle. For the first time, we showed that ABP1 plays a critical role at both the $G_1$/S checkpoint and the $G_2$/M transition. Furthermore, cell cycle arrest provoked by ABP1 inactivation cannot be bypassed by exogenous auxin suggesting that ABP1 mediates the auxin control of the cell cycle.

How do these data fit with what is known concerning auxin action at the plant level? Characterisation of the *abp1* knock-out mutant indicates that the protein is involved in both cell division and cell expansion during embryonic development. In a mature plant, auxin-mediated cell expansion occurs in cells which exit the cell cycle and enter into differentiation.[4] Could ABP1 be involved sequentially in the auxin control of cell division then in cell expansion? In a cell system such as the BY2 cell suspension culture, the same cells alternatively divide and elongate. With a drastic inactivation of ABP1 resulting in a rapid cell cycle arrest, we could detect no other effect whereas in the case of a partial ABP1 suppression via constitutive antisense, cell division was maintained, thus revealing a defect in cell expansion.[5] ABP1 can thus act on both cellular responses depending on the cell type or stage of development. One of the challenges is now to determine the role of ABP1 at the whole plant level and during plant growth.

ABP1 is not the sole auxin receptor as an auxin receptor function has recently been assigned to the F-box protein TIR1 and related AFB proteins.[7-9] The existence of two auxin signalling pathways raises the question of the respective role of ABP1 and TIR1 in the processes of cell division and cell expansion. Through its effect on the degradation of IAA/Aux repressors and regulation of early auxin responsive genes, TIR1 is potentially involved in a large range of auxin cellular responses.[7,10] Many *IAA/Aux* genes are expressed in elongating cells, correlating TIR1 activity with elongation processes. Other members of the *IAA/Aux* family however are associated with cell division; e.g., *IAA14/SLR* which is involved in lateral root initiation.[11] Is the respective role of TIR1 and ABP1 dependent of the tissue, organ, or developmental stage? In a near future, detailed analysis of ABP1 function in plants may help to understand the respective role of both receptors in auxin mediated growth responses.

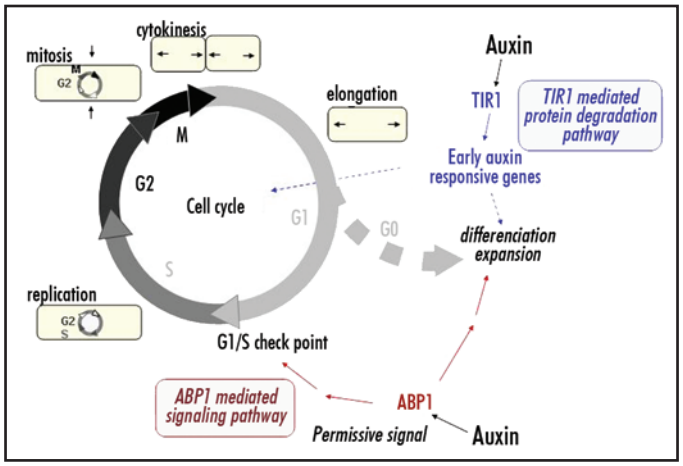


Figure 1. Illustration of the dual roles of the ABP1 and TIR1 auxin receptors in control of the cell cycle. ABP1 has been demonstrated to mediate the permissive auxin signal for entry into the cell cycle and to modulate the $G_2$/M transition.[15] Plants defective in ABP1 also show aberrant cell expansion.[4,5] A number of genes thought to be controlled by the TIR1 mediated protein degradation pathway have been implicated in various auxin responses.[10]

## CELLULAR IMMUNIZATION: AN EFFICIENT ALTERNATIVE TO CLASSICAL GENETIC APPROACHES TO ADDRESS PROTEIN FUNCTION

Many approaches have been taken in investigating the function of ABP1 and researchers have faced a lot of difficulties due to the proteins low abundance, dual location and a tightly controlled but unknown mechanism of ABP1 targeting at the plasma membrane. Progress has also been hindered by the lethality of the *abp1* mutant and lack of phenotype in the heterozygote.[12] Acting at the protein level via the use of a recombinant antibody appeared an attractive means to manipulate the activity of ABP1 in vivo. Such cellular immunization approaches are based on the in vivo expression of recombinant immunoglobulin fragments termed scFv (single chain Fragment variable) which consist of the heavy and light chain variable domains linked by a flexible peptide.[13] This is the minimum antibody form still retaining specificity and monovalent binding affinity of the full-size parent antibody. In our study we used an scFv12 derived from the well characterised and high affinity monoclonal antibody mAb12 which has been shown to block ABP1 in an inactive conformation.[1]

Whilst this technique has been used to modulate the activity of endogenous proteins of known function in plant and animal cells[14] we report its first use to address the role of a given protein. Mini-antibodies present an attractive alternative to knock-out and antisense strategies. scFv can be used to directly inhibit an enzymatic activity, to interfere with protein-protein interactions or protein-ligand interaction or to disrupt protein targeting, thus disturbing protein activity or signalling pathways. A potentially interesting feature of cellular immunization is also that scFv can be addressed to distinct cellular compartments (nucleus, cytosol, mitochondria…), thus targeting protein interference to specific subcellular domains. The key element for a successful approach lies in the choice of the antibody, in particular the characteristics of the interaction with the target, its affinity and specificity. It is also important to confirm that the recognition and binding to the target are not altered in the scFv construct and that the fragment produced in plant cells recognises the endogenous target protein. Increased availability of scFv-phage display libraries now offers broader possibilities to easily identify specific recombinant antibodies against a protein of interest. Characterising their interaction with the antigen prior to any further use however, remains an essential step. We have demonstrated that cellular immunization is an efficient approach to impair protein activity and to investigate protein function even for a low abundant and essential protein.

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