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SCIENTIFIC CORRESPONDENCE

Plant Cells Are Not Just Green Yeast

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Are plant cells just yeast cells with chloroplasts? Should plant cell biologists who don't work on chloroplasts just switch to yeast to help solve the basic questions that are common to all eukaryotes? The recent completion of the sequencing of the entire yeast (*Saccharomyces cerevisiae*) genome and the availability of hundreds of well-characterized mutants certainly make this a tempting proposition. At the same time, these tools provide unprecedented opportunities for plant biologists. We can exploit these resources, but there is already enough evidence to interpret the results with caution, because plant cells are not just green yeast.

The isolation of plant genes by screening cDNA expression libraries for complementation of yeast mutant phenotypes is a valuable technique for identifying gene products that have a specific biochemical activity or are involved in a particular pathway. In addition, the reverse process, testing a previously identified plant gene with sequence similarity to a yeast gene for complementation of the corresponding yeast mutant, has allowed a function to be assigned to the gene product in cases where discovering function in the plant would have been extremely difficult. However, recent data from our laboratory and from other investigators illustrate the need to carefully study protein localization and function in plants rather that relying solely on the results obtained with yeast, as this may be misleading. Plant proteins may not always be localized correctly when expressed in yeast, particularly when overexpressed from a multicopy plasmid, and the specificity of a number of proteins may be dependent on subcellular compartmentation. Care must therefore be taken when analyzing data from yeast expression, and the information obtained with yeast needs to be confirmed as much as possible in the plant.

Traditional biochemical approaches in general were unsuccessful in the isolation of genes encoding transporters and channels, whereas yeast complementation enabled a wide variety of different genes to be isolated. For example, the screening of Arabidopsis cDNA libraries for the complementation of two different yeast amino acid transport mutants led to the isolation of the same gene encoding an amino acid permease (NAT2/AAP1; Frommer et al., 1993; Hsu et al., 1993). The Arabidopsis gene is unrelated in sequence to the yeast gene, but clearly the protein has a related transport activity. Similarly, screening for complementation of a yeast mutant defective in K^+ uptake led to the isolation of three different Arabidopsis genes encoding putative K^+ transporters: AKT1 (Sentenac et al., 1992), KAT1 (which is similar in sequence to AKT1 but not allelic; Anderson et al., 1992), and HKT1 (which is unrelated to AKT1/KAT1; Schachtman and Schroeder, 1994). The yeast cells also provide a convenient system for uptake studies using these and other transporters, and have been useful for investigating their transport mechanism and specificity. However, the activity of the transporters in plants may be modulated by their interactions with other proteins that are absent in yeast, and the expression of the genes may be developmentally or environmentally regulated, which may contribute to their specific functions. In addition, the transporters are in general present at the plasma membrane in yeast, and it is assumed that the same is true in plants. This needs to be addressed for each protein individually, as the plant cell has a more complex endomembrane system that presumably also contains a variety of transport activities, and some of these could be mistargeted to the plasma membrane upon expression in yeast.

One example of a transporter that appears to be localized differently in yeast and plants has been described recently (Apse et al., 1999; Gaxiola et al., 1999). AtNHX1 from Arabidopsis is a member of a family of intracellular Na^+/H^+ exchangers and was identified based on sequence similarity to yeast Nhx1. In yeast, Nhx1 is found on a prevacuolar compartment (PVC) and is involved in salt tolerance by mediating Na^+ sequestration in the PVC. The *AtNHX1* cDNA is able to complement some of the phenotypes of the yeast *nhx1* mutant (Gaxiola et al., 1999), and overexpression of *AtNHX1* in Arabidopsis conferred salt tolerance on the transgenic plants (Apse et al., 1999). However, subcellular localization of the protein in plants indicated that it is in fact a tonoplast protein (Apse et al., 1999), and its ability to complement the yeast mutant may reflect its mislocalization to the PVC upon heterologous expression.

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We have used yeast mutant complementation extensively in studying vesicular transport in Arabidopsis, both to isolate components of the transport machinery involved in clathrin-coated vesicle-mediated transport to the vacuole, and to test the function of genes identified in the various Arabidopsis sequencing projects. The requirements for complementation of vesicle trafficking mutants are expected to be very stringent, as the plant protein not only has to maintain its biochemical activity in yeast, but also must interact with a complex array of yeast proteins to function correctly. In light of this, it is perhaps surprising that Arabidopsis genes have been found to complement yeast secretory and vacuolar transport mutants in a number of different cases, allowing the tentative assignment of functions to these proteins. In some cases, the available evidence still suggests that the yeast and Arabidopsis proteins have equivalent functions: e.g. the ER-to-Golgi trafficking proteins Sar1p and Sec12p (d'Enfert et al., 1992) and the prevacuolar t-SNARE (SNAP receptor; a protein found on the target membrane required for vesicle fusion with that membrane) Pep12p (Bassham et al., 1995). However, several examples have come to light recently in which the correlation between localization and/or function of protein trafficking machinery in yeast and plants is not so clear.

One example is the Arabidopsis protein designated AtVAM3. The *AtVAM3* gene was isolated because of its ability to complement several phenotypes of the yeast *vam3* mutant (Sato et al., 1997). Yeast Vam3p is a t-SNARE present in the vacuolar membrane, where it functions in the fusion of transport vesicles from several trafficking pathways with the vacuole. The Arabidopsis protein was also initially reported to be located on the tonoplast in shoot apical meristems (Sato et al., 1997). However, further examination of the localization of AtVAM3 in different tissues by gradient fractionation and by immunoelectron microscopy using both specific antibodies and epitope tagging demonstrated that in roots and leaves, AtVAM3 is found on the PVC, where it co-localizes with AtPEP12, and is not detectable on the tonoplast (Sanderfoot et al., 1999). AtVAM3 therefore is probably involved in vesicle transport from the trans-Golgi network to the PVC or in the fusion of prevacuoles, rather than in the transport from the PVC to the vacuole, at least in roots and leaves. It is likely that complementation of the yeast mutant reflected the mistargeting of AtVAM3 due to differences in the endomembrane system and in protein targeting between yeast and plants. From these data, we conclude that: (a) the localization of a protein in yeast does not necessarily correspond with the localization of that protein in its native plant species (see Fig. 1); and (b) proteins can reside on different organelles in different cell types within the same species, possibly due to differences in the endomembrane structure between cell types.

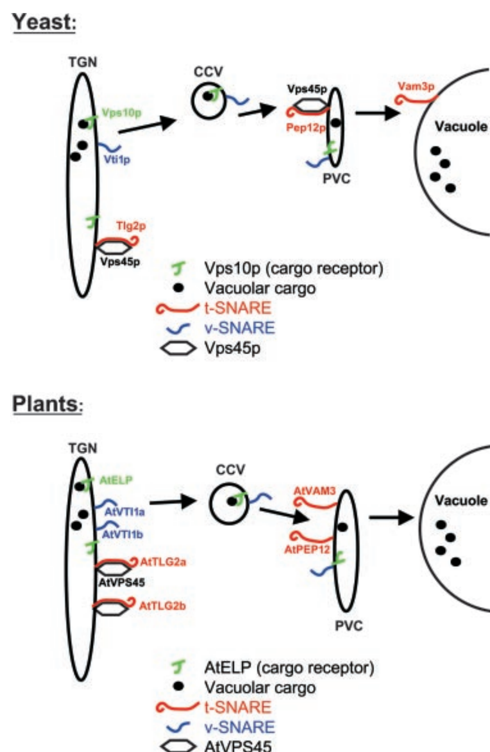

Figure 1. Comparison of the localization of proteins thought to be involved in clathrin-coated vesicle-mediated transport to the vacuole in yeast and plants. Note that two of the Arabidopsis proteins shown have differences in localization compared with yeast. AtVAM3 is found on the PVC in Arabidopsis, whereas yeast Vam3p is found on the vacuole. AtVPS45 is present only at the TGN, whereas yeast Vps45p is localized to both the TGN and the PVC.

Another observation made in the study of the plant secretory pathway is that two or more Arabidopsis genes may appear to correspond to a single gene in yeast. One example is that of the yeast *PEP12* gene mentioned above. At least three genes in Arabidopsis are closely related in sequence to yeast *PEP12*: *AtPEP12* (Bassham et al., 1995), *AtVAM3* (Sato et al., 1997), and *AtPLP* (Zheng et al., 1999a). It is not clear whether this represents a specialization of function or simply redundancy between these three genes. However, our preliminary observations suggest that AtPEP12 and AtVAM3 do not perform redundant functions.

An intriguing case of multiple Arabidopsis versions of a single yeast gene is that of the vesicle-SNARE *VTI1*. Yeast Vti1p is a multifunctional protein involved in vesicle fusion in several different transport pathways. There are *vti1* mutant alleles that are defective in only a subset of the pathways and are able to function normally in others. Two Arabidopsis genes related to *VTI1* (*AtVTI1a* and *AtVTI1b*) have

been identified and the various yeast *vti1* mutant alleles used to address their function (Zheng et al., 1999b). The AtVTI1a and AtVTI1b proteins function in distinct transport pathways when expressed in the yeast mutants, as they complement the phenotypes of different mutant alleles. This suggests that each Arabidopsis protein may be specialized for a particular subset of functions of yeast Vti1p; however, this hypothesis remains to be tested in plants.

A final example of different functions observed between yeast and plant genes is the Sec1p-like protein Vps45p. In yeast, this protein functions in two transport pathways to the vacuole: (a) the carboxypeptidase Y pathway, where it is involved in fusion of TGN-derived vesicles at the PVC and interacts with the prevacuolar t-SNARE Pep12p; and (b) the cytoplasm-to-vacuole pathway, where it is required for the formation of transport intermediates at the TGN and interacts with the TGN t-SNARE Tlg2p. An Arabidopsis cDNA encoding a protein (AtVPS45) with extensive sequence similarity to yeast Vps45p is able to complement the carboxypeptidase Y sorting defects and the temperature-sensitive growth defect of a yeast *vps45* mutant (Bassham and Raikhel, 1998). However, AtVPS45 in Arabidopsis resides exclusively on the TGN, not on the PVC, and interacts only with the TGN t-SNAREs and not with t-SNAREs found on the PVC (D.C. Bassham, A.A. Sanderfoot, V. Kovaleva, H. Zheng, and N.V. Raikhel, unpublished data). It therefore appears that AtVPS45 can perform functions in yeast (vesicle fusion at the PVC) that it does not normally perform in the plant. We hypothesize that a different protein substitutes for AtVPS45 at the Arabidopsis prevacuole, but this protein has yet to be identified.

Presented above are just a few examples in which expression in yeast has been used to isolate or characterize plant genes. Overall, yeast mutants have been found to be extremely useful tools for rapidly identifying genes encoding proteins functioning in a particular pathway or with a specific biochemical activity, and many advances have been made with this approach. We have tried to draw attention to some of the limitations to this experimental approach, not to imply that yeast complementation is not useful, but rather to suggest that it is only a starting point. It is now clear that the localization and function of proteins need to be investigated in plants before any firm conclusions can be drawn. The completion of the genome sequence of Arabidopsis and the availability of knockout mutants will make this possible for a wide range of genes that would otherwise be very difficult to analyze in plants.

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