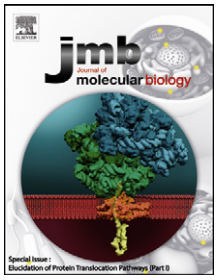

Experimental Validation of Plant Peroxisomal Targeting Prediction Algorithms by Systematic Comparison of *In Vivo* Import Efficiency and *In Vitro* PTS1 Binding Affinity

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Abstract

Most peroxisomal matrix proteins possess a C-terminal targeting signal type 1 (PTS1). Accurate prediction of functional PTS1 sequences and their relative strength by computational methods is essential for determination of peroxisomal proteomes *in silico* but has proved challenging due to high levels of sequence variability of non-canonical targeting signals, particularly in higher plants, and low levels of availability of experimentally validated non-canonical examples. In this study, *in silico* predictions were compared with *in vivo* targeting analyses and *in vitro* thermodynamic binding of mutated variants within the context of one model targeting sequence. There was broad agreement between the methods for entire PTS1 domains and position-specific single amino acid residues, including residues upstream of the PTS1 tripeptide. The hierarchy Leu>Met>Ile>Val at the C-terminal position was determined for all methods but both experimental approaches suggest that Tyr is underweighted in the prediction algorithm due to the absence of this residue in the positive training dataset. A combination of methods better defines the score range that discriminates a functional PTS1. *In vitro* binding to the PEX5 receptor could discriminate among strong targeting signals while *in vivo* targeting assays were more sensitive, allowing detection of weak functional import signals that were below the limit of detection in the binding assay. Together, the data provide a comprehensive assessment of the factors driving PTS1 efficacy and provide a framework for the more quantitative assessment of the protein import pathway in higher plants.

Introduction

Peroxisomes are ubiquitous organelles within eukaryotes, responsible for a wide range of intracellular roles that are critical to cell and organism function. Compared to other cell organelles, peroxisomes are very dynamic and metabolically versatile. For example, in cotyledons of *Arabidopsis thaliana* and other oil seed plants, a major role of peroxisomes is in mobilization of storage lipids and conversion into carbohydrates to support early heterotrophic seedling growth. As the cotyledons become photoautotrophic, photorespiration becomes the predominant pathway. Additionally, it is increasingly apparent that peroxisomes are connected into many if not all aspects of plant life, including primary metabolism, hormone synthesis and signaling of reactive oxygen species [1]. Proteomic studies from different tissues reveal new and unexpected peroxisomal capabilities, for example, in synthesis of secondary metabolites and in plant defense [2–5]. Collectively, these roles are of critical importance for plant fitness and productivity, underscored by the severe, sometimes lethal, phenotypes of peroxisome biogenesis mutants [6,7]. Different peroxisome functions are determined

by their precise enzyme set that, in turn, reflects the balance between import and turnover of individual proteins and the organelle as a whole [1].

Proteins destined for the peroxisomal matrix are typically synthesized in the cytosol with one of two peroxisome targeting signals (PTS1 or PTS2) within their sequence. These are recognized by cytosolic receptors that initiate the import of the cargo protein into the peroxisome. The peroxisome targeting signal type 1 (PTS1) was initially described as a

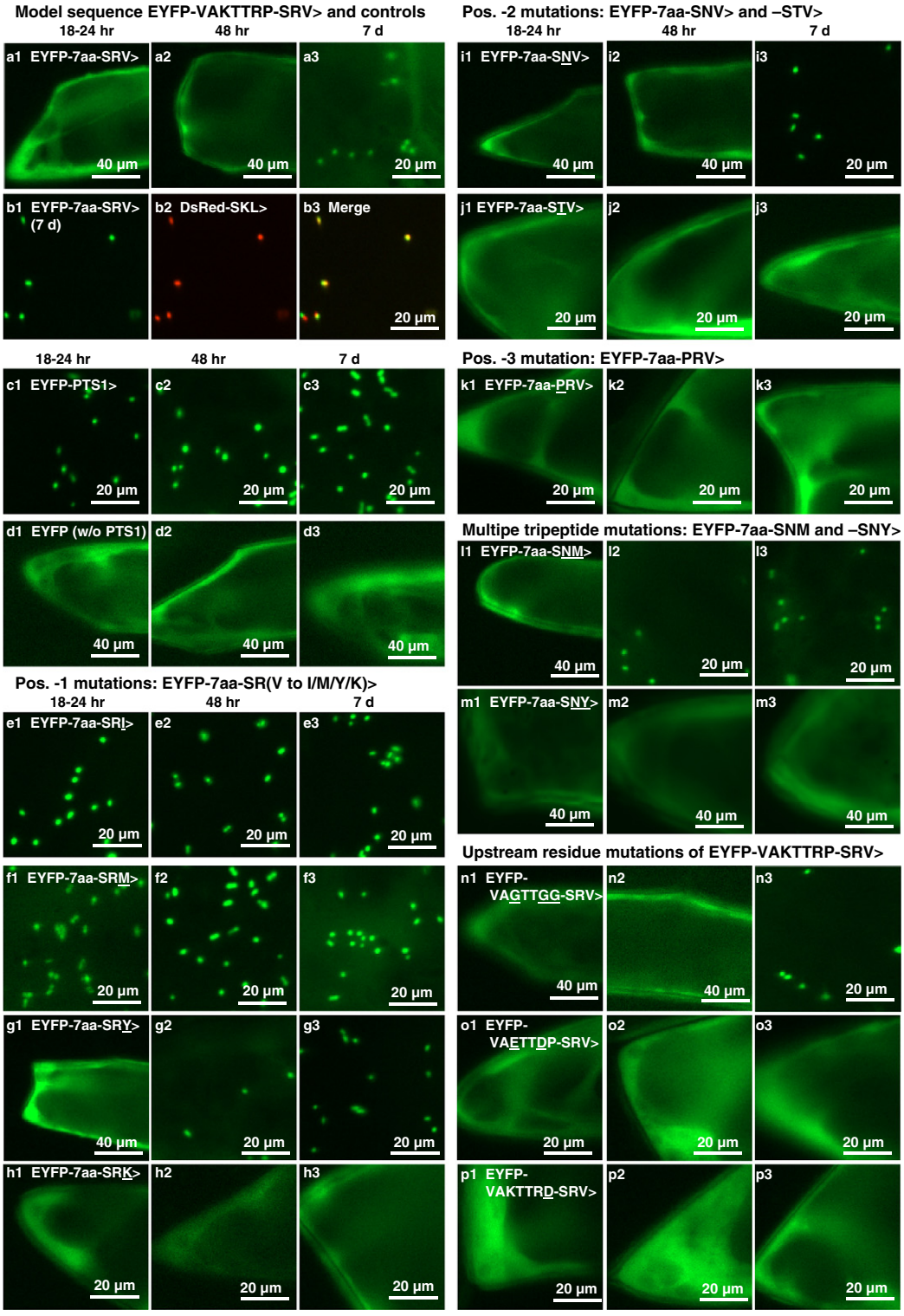

Fig. 1 (*legend on next page*)