1-1-2010

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Recommended Citation

Kato, N. (2010). A systems model of exocytic vesicles in Arabidopsis pollen during its germination. *Plant Signaling and Behavior, 5* (4), 464-466. https://doi.org/10.4161/psb.5.4.10911

Plant Signaling & Behavior

ISSN: (Print) 1559-2324 (Online) Journal homepage: https://www.tandfonline.com/loi/kpsb20

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To cite this article: Naohiro Kato (2010) A systems model of exocytic vesicles in Arabidopsis pollen during its germination, Plant Signaling & Behavior, 5:4, 464-466, DOI: 10.4161/psb.5.4.10911

To link to this article: https://doi.org/10.4161/psb.5.4.10911

Published online: 01 Apr 2010.

Plant Signaling & Behavior 5:4, 464-466; April 2010; © 2010 Landes Bioscience

A systems model of exocytic vesicles in Arabidopsis pollen during its germination

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A basic systems model for vesicle trafficking in Arabidopsis pollen tubes was constructed. The model was composed of transcriptome data and differential equations. The transcriptome data revealed some genes controlling vesicle trafficking in the pollen tubes, and the differential equations connected the molecular functions of the gene products. The computed pollen tube growth reasonably agreed with biological samples. Here, I expand the computational prediction into exocytic vesicles during pollen germination, which can be used to examine the accuracy of the systems model with biological samples. The computational analysis of the model predicts that the number of exocytic vesicles changes in an over 10-fold range before the vesicle trafficking system reaches the equilibrium that makes the pollen tube grow logarithmically. SYP125 (syntaxin of plants 125) is highly localized in the pollen tube tip in both the biological sample and systems model. The computational analysis predicts that SYP125 would highly localize in exocytic vesicles temporally before the pollen tube grows logarithmically. These kinetic predictions guide future research directions.

Key words: systems biology, pollen tube, arabidopsis, mathematical model

Submitted: 12/08/09

Accepted: 12/09/09

Previously published online: www.landesbioscience.com/journals/psb/article/10911

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Addendum to: Kato N, He H, Steger AP. A systems model of vesicle trafficking in Arabidopsis pollen tubes. Plant Physiol 2009; 152:590-601; PMID: 19933386; DOI: 10.1104/pp.109.148700.

One of the goals of systems biology is to construct a mathematical model that is used to predict a system output in biological samples.[1] Because we are interested in controlling vesicle trafficking in plant cells through genetic modification, we sought to construct a mathematical model for vesicle trafficking in plant cells.[2] However, in the early stage of the construction, we noticed that even if we were able to construct a mathematical model for vesicle trafficking in cells of interest, it would be difficult to genetically evaluate the model due to functional redundancy of paralogous genes expressed within the cells.[3] Therefore, we focused our study on Arabidopsis pollen. We expected Arabidopsis pollen might express much fewer numbers of paralogous genes that control vesicle trafficking (vesicle trafficking genes), compared to other cell types, because of overall reduction of the number of genes expressed.[4-6] Hence, the importance of the transcriptome analysis in this study is to make the mathematical model testable through genetic analyses. The original goal of transcriptome analysis was to identify genes within the same gene families expressed in pollen. Our analysis suggested that only selected genes within the gene families are expressed in pollen as expected.[7] Moreover, within the vesicle trafficking genes enriched or selectively expressed in pollen, we unexpectedly found a group of genes that are clearly downregulated in pollen of the gibberellin (GA) pathway mutant lines.[7] This suggested that the GA signaling pathway may control vesicle trafficking in pollen. Based on the transcriptome data, we constructed a mathematical model that connects the molecular functions of the genes that are enriched or selectively expressed in Arabidopsis pollen.[7] The current model is limited to connect the functions of 14 molecules; 6 SNAREs (N-ethylmaleimide sensitive factor attachment protein receptors), 6 small GTPases (guanosine triphosphatases; Arf or Rab), pectin and phospholipid. Our comparative analysis suggested that the model is capable of

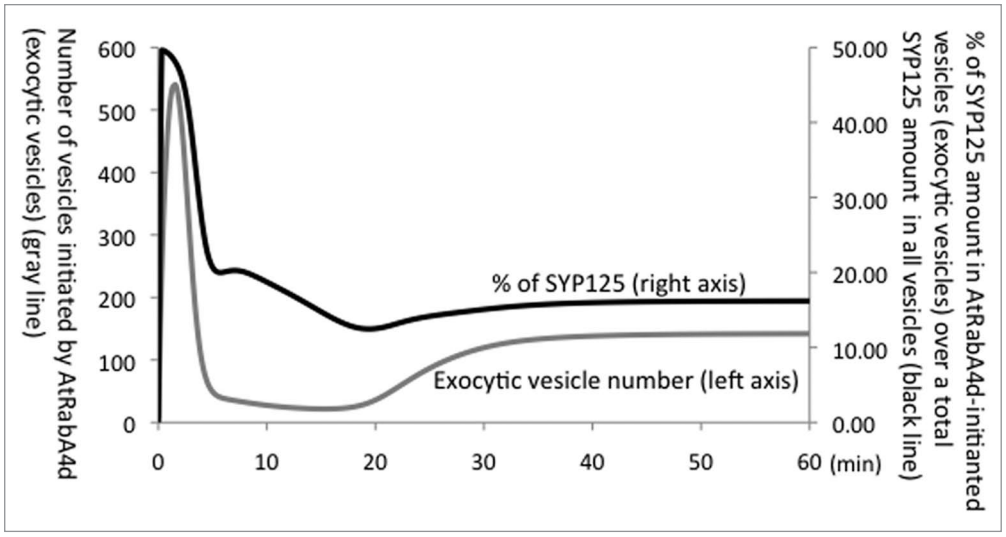

Figure 1. Changes in SYP125 recruitment to exocytic vesicles initiated by AtRabA4d. The computational prediction of changes in a number of vesicles initiated by AtRabA4d after the initiation of the tube (gray line). The vesicle number is the highest (513 vesicles) at 2 min, but rapidly reduced to 46 vesicles at 5 min. At the equilibrium (after 47 min), the number is stable at 142 vesicles. On the other hand, the percentage of SYP125 amount carried by the vesicles over a total amount of SYP125 in all vesicles in the system reaches the highest (49%) 30 sec after the initiation (black line). The percentage is reduced to 16% at the equilibrium.

predicting the pollen tube growth and subcellular localizations of selected molecules in both wild type and selected gene-knockout mutant lines reasonably.[7] Further analysis is required to verify the model before expanding the model to connect more molecules present in pollen.

The result presented in this article is a guideline to determine how accurately our hypothesis has been translated into mathematical form. If any difference is identified, the model must be modified accordingly. The computation analysis of our current model can predict the number of exocytic vesicles and SYP125 (syntaxin of plants 125) localization on the exocytic vesicles that are initiated by AtRabA4d, small GTPase selectively expressed in pollen, during pollen germination (at the stage of pollen tube formation) (**Fig. 1**). It predicts that vesicle trafficking reaches equilibrium 47 min after pollen germination. However, before vesicle trafficking reaches equilibrium, the number of exocytic vesicles changes dynamically. The number of the vesicles is increased from 7 to 513 vesicles in the first 2 min after the germination, but rapidly reduced to less than 50 vesicles at 5 min (**Fig. 1**). This lower level lasts for 15 min. After this point, the number increases logarithmically until it reaches equilibrium of 142 vesicles. In the systems model, we assume that SYP125 accumulated in the Golgi apparatus at the initiation of the tube tip is transported to the tube tip through either exocytosis (vesicle trafficking from the Golgi apparatus to the tube tip) or recycling pathway (vesicle trafficking from the recycling endosome to the tube tip). Hence, the computational analysis predicts that a percentage of SYP125 in exocytic vesicles that are initiated by AtRabA4d over a total SYP125 amount in all vesicles (including vesicles generated by endocytosis and recycling endosome) changes dynamically. The percentage of SYP125 in the exocytic vesicles over the total SYP125 in all vesicles increases from 0 to 49% in the first 30 sec after the pollen germination, but rapidly reduces to 12% at 20 min (**Fig. 1**). After this point, the percentage of SYP125 slowly and slightly increases to 16% at 47 min, when it reaches equilibrium.

Enami et al. previously shows localization of GFP (green fluorescent protein)-SYP125 that is expressed from its own promoter in wild type Arabidopsis pollen.[8] Although the GFP signaling is highly intense in the plasma membrane of the tube tip, GFP signals are not detected in the plasma membrane specifically, but rather the signals appear as granules in the cytosol nearby the tube tip and in pollen that do not form the tube yet.[8] On the other hand, Szumlanski et al. previously shows localization of YFP (yellow fluorescent protein)-AtRabA4d in *atraba4d* knockout pollen.[9] Similar to GFP-SYP125, YFP-AtRabA4d signals also appear as granules in the cytosol nearby the tube tip and in pollen that do not form the tube yet.[9] We expect that these granules are exocytic vesicles initiated by AtRabA4d upon the pollen germination.[7] By conducting a quantitative co-localization assay of GFP-SYP125 and YFP-RabA4d in pollen with a function of time, the accuracy of the systems model should be evaluated and allow preliminary estimation of the empirical parameters toward expansion of the model. Namely, the constants for the budding and recruitment rates may be preliminarily estimated by combining the results with previously published biological data about a SNARE amount (approximately 830,000 molecules per cell)[10] and an average ratio of SNARE to small GTPase in a vesicle (approximately 8:1),[11] or by measuring the values in pollen. If no co-localization is found in the exocytic vesicles, we can conclude that SYP125 is not recruited to exocytic vesicles by AtRabA4d nor recycled during pollen tube growth.

Acknowledgements

N.K. thanks Irene M. Bognar for her critical reading.

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