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Plants Get Hyp to O-Glycosylation

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autophagy did not last more than 6 to 8 hours into starvation. Recent studies, however, suggest that it can continue for days, with the degradation process shifting from proteins to more energetically favorable cargos, such as intracellular lipids (*11*), over time.

How are cells sustaining autophagy over these longer periods? Recycling of Atgs is one possibility. Some of the structural components of the autophagosome, for example, are recycled back to the cytosol before they fuse with lysosomes (*3*, *5*). This recycling also applies to the lysosomal compartment itself. During starvation, the vast increase in autophagosome formation often means that all existing lysosomes are engaged in fusing with newly formed autophagosomes. As starvation persists, cells also actively recycle components of the lysosomal membrane out of the hybrid vesicles (autophagolysosomes) (*12*). But most lysosomal enzymes—which are the ones that get the degradative job done—are not retrieved out of the autophagolysosomes. As a result, new synthesis of lysosomal hydrolases may be necessary to transform recycling vesicles into functional lysosomes. The activation of TFEB during starvation provides a solution for both Atg consumption and the need for new lysosomes.

Other transcriptional regulators increase the expression of Atgs, but often only those Atgs involved in the early steps of autophagosome formation (*13*, *14*). The strength of the TFEB-mediated program is that it affects the whole process; it not only generates more autophagosomes, but also accelerates their delivery to lysosomes and, by increasing the number of available lysosomes, facilitates the rapid degradation of substrates. This aspect of the autophagy process is often overlooked. Forming autophagosomes and secluding the materials from the cytosol is not enough. The ultimate purpose of autophagy is to break down the cargo and recycle essential macromolecules, and this only occurs once the lysosomal hydrolases reach the autophagosome through fusion.

Defective autophagy has been linked to common human diseases such as neurodegenerative conditions (e.g., Alzheimer's disease, Parkinson's disease), metabolic disorders (diabetes, obesity), and aging. The formation of autophagosomes is intact or even enhanced in many of these pathologies; it is the failure to degrade these structures that compromises cellular viability (*15*). Pharmacological interventions have succeeded in enhancing autophagosome formation by suppressing negative regulators. The main concern about this approach, however, is that it could lead to an "autophagic traffic jam" if the cell does not have enough lysosomes to receive all the cargo. The ability of TFEB to control the formation of both lysosomes and autophagosomes makes it a very attractive target for developing new therapies for those conditions in which enhanced autophagy is desirable.

References and Notes

1. Z. Yang, D. J. Klionsky, *Nat. Cell Biol.* **12**, 814 (2010).
2. N. Mizushima, A. Yamamoto, M. Matsui, T. Yoshimori, Y. Ohsumi, *Mol. Biol. Cell* **15**, 1101 (2004).
3. Z. Yang, D. J. Klionsky, *Curr. Opin. Cell Biol.* **22**, 124 (2010).
4. C. Settembre *et al*., *Science* **332**, 1429 (2011); 10.1126/science.1204592
5. K. Suzuki, Y. Ohsumi, *FEBS Lett.* **584**, 1280 (2010).
6. T. Lamark, V. Kirkin, I. Dikic, T. Johansen, *Cell Cycle* **8**, 1986 (2009).
7. J. L. Webb, B. Ravikumar, D. C. Rubinsztein, *Int. J. Biochem. Cell Biol.* **36**, 2541 (2004).
8. J.-Y. Lee *et al*., *EMBO J.* **29**, 969 (2010).
9. M. Razi, E. Y. Chan, S. A. Tooze, *J. Cell Biol.* **185**, 305 (2009).
10. M. Sardiello *et al*., *Science* **325**, 473 (2009).
11. R. Singh *et al*., *Nature* **458**, 1131 (2009).
12. L. Yu *et al*., *Nature* **465**, 942 (2010).
13. D. A. M. Salih, A. Brunet, *Curr. Opin. Cell Biol.* **20**, 126 (2008).
14. P. Xu, M. Das, J. Reilly, R. J. Davis, *Genes Dev.* **25**, 310 (2011).
15. E. Wong, A. M. Cuervo, *Nat. Neurosci.* **13**, 805 (2010).

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PLANT SCIENCE

Plants Get Hyp to O-Glycosylation

The polarized growth of plant root hair cells requires specific glycosylation of proteins in the plant cell wall.

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The two most abundant natural organic polymers on Earth are cellulose and chitin, characterized by long chains of carbohydrates that bear a specific type of sugar linkage called O-glycosylation. This type of linkage also occurs between polysaccharides (glycans) and proteins and glycans and lipids, yielding glycoconjugates that are well known to function in cell recognition processes (*1*). On page 1401 in this issue, Velasquez *et al*. (*2*) explore a specific type of O-glycosylation for plant cell wall structural proteins and connect this modification to root hair growth.

Two types of sugar linkages predominate in glycoproteins: N-linkage of glycans to asparagine residues, and O-linkages, which are structurally more complex and most commonly connect glycans to the hydroxyl group of serine or threonine residues. Glycans, however, can also be attached to lysine or proline (Pro) if these amino acids are first hydroxylated. This type of O-glycosylation is addressed by Velasquez *et al*.

Hydroxyproline (Hyp) is prevalent in animal extracellular matrix structural proteins such as collagen, and in hydroxyproline-rich glycoproteins (HRGPs) such as those found in the plant cell wall. Hyp, however, also occurs in regulatory proteins such as Argonaute 2 in RNA silencing (*3*), the transcription factor HIF-1α (*4*), Cle peptides that control plant cell differentiation (*5*), and hypsystemins that signal for plant defense (*6*). The enzymes that catalyze Pro hydroxylation include prolyl 4-hydroxylases (P4Hs) (*7*). The conversion of Pro to Hyp affects protein conformation and protein-protein interactions, and provides reactive hydroxyl groups for further modification such as glycosylation. The model plant *Arabidopsis thaliana* encodes 13 P4Hs, but only P4H1 (*8*) and P4H2 (*9*) have been characterized at the molecular level. *Arabidopsis* has at least 166 HRGP superfamily members, many of which are differentially expressed during plant growth. The extent of HRGP prolyl hydroxylation can be predicted (*10*), and establishes the HRGP glycosylation profile.

Velasquez *et al*. focused on HRGP function in *Arabidopsis* roots hairs (see the figure), tractable cells that elongate by polarized growth. Inhibition of P4H activity blocked root hair growth and reduced the O-glycosylation of an extracellular matrix HRGP. The authors identified three P4Hs that are highly expressed in root hair cells (P4H2, 5, and 13), and observed that the corresponding mutants exhibited reduced root hair length, a decrease in total root Hyp content and, for P4H2, reduced root hair

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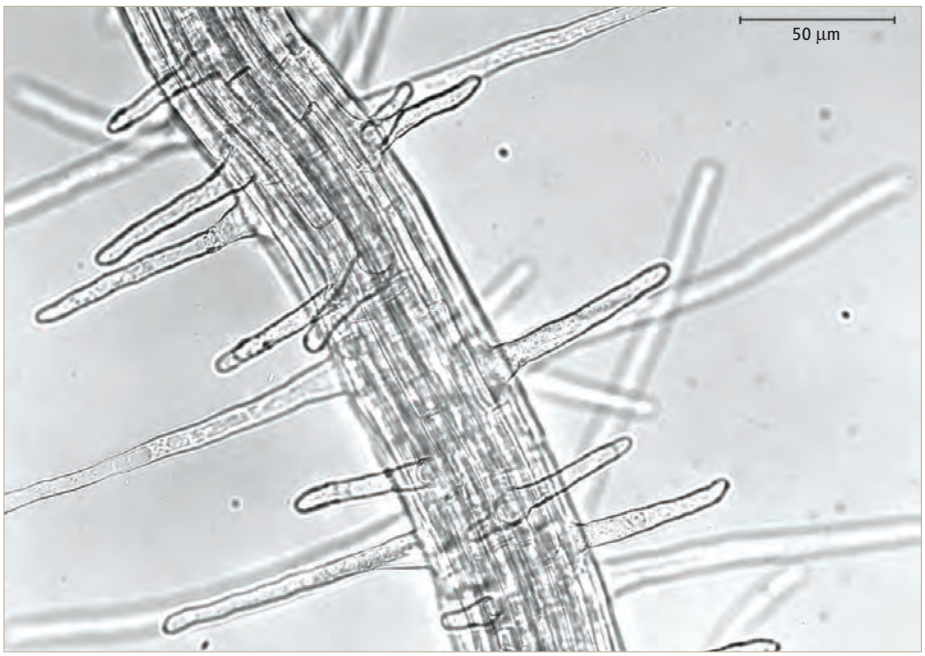

P4Hs tip the scale for root hair growth. Prolyl 4-hydroxylases (P4Hs) are required for plant root hair growth (shown are *Arabidoposis* root hairs) and provide a molecular model of extracellular matrix assembly in which the proline hydroxylation (Hyp) of extensin, a hydroxyproline-rich glycoprotein (HRGP), is required for function in the plant cell wall. This opens the door to examining mechanisms through which Hyp modification of regulatory and signaling proteins for HRGPs controls plant growth and function.

density. Overexpression of these hydroxylases individually in plants had the opposite effect. These results agree with the phenotype of *Arabidopsis* lines in which hypoxia-induced overexpression of P4H1 increases Hyp content and root hair length and density (*11*). Velasquez *et al*. do not discuss hypoxia-associated P4H1 or the comparable overexpression phenotype in their study, but concentrate on the premise that the root hair phenotype observed in the P4H2, 5 and 13 mutants is due to alterations in the posttranslational modification of Hyp in one or more HRGP proteins in root hairs.

Through a yeast two-hybrid system approach, Velasquez *et al*. identified seven candidate proteins that interact with P4H5, including a glycine-rich cell wall protein and an extension protein called LRX3, a HRGP cell wall constituent. Homology modeling of P4H2, 5, and 13 each with possible peptide substrates suggested a preference for poly-Pro-like substrates and for extensins, indicating that the absence of O-glycosylation of extensins in the P4H mutant plants could be responsible for the root hair phenotypes.

Extensins are proposed to assemble into extensive networks within the plant cell wall (*12*) and contain repetitive units, each comprising four to six Hyp residues that are often glycosylated with short arabinose side chains (*10*). Velasquez *et al*. identified two putative arabinosyltransferases [XEG113 (*13*) and reduced residual arabinose 3 (RRA3)] that are transcribed coordinately with P4H2 and P4H5. RRA3 is homologous to RRA1 and RRA2, which have been implicated in extensin glycosylation (*14*, *15*). Mutant plants failing to express XEG113 or RRA3 had shorter root hairs, similar to the P4H2, 5 and 13 mutants. Velasquez *et al*. conclude that the P4Hs hydroxylate extensins, which are then arabinosylated. Mass spectrometry data show that peptides released from *rra3* and *xeg113* mutants have shorter oligoarabinoside side chains, strongly suggesting that RRA3 and XEG113 incorporate arabinose into the oligosaccharide side chains of extensins. Although these results require confirmation of RRA3 and XEG113 enzymatic activity, they provide new information on the roles of these putative arabinosyltransferases in HRGP glycosylation.

Velazquez *et al*. establish a role for three P4Hs in root hair elongation in *Arabidopsis* and two putative arabinosyltransferases involved in the O-glycosylation of HRGPs. However, the link between the P4H mutant root hair phenotypes and the root hair phenotypes of the other mutants will require further characterization of P4H substrates. An expanded model in which P4Hs also modify regulatory or signaling proteins contributing to root hair growth deserves consideration. In addition, if cell wall structure is being altered in root hairs due to a change in the biochemical modification of extensins, it is likely that this alone may result in altered gene expression in root hairs and that those changes may also contribute to the root hair phenotype observed in many of the mutants characterized here.

The similarity of the mutant phenotypes of the three P4Hs (P4H2, 5, and 13) with those of P4H1, which has been associated with hypoxia stress responses in plants (*11*), raises the question of how similar the molecular mechanism of root hair growth is for these four P4Hs. The overexpression of P4H1 in *Arabidopsis* leads to dramatic changes in the expression of over 400 genes including cell wall, lipid, protein, secondary product, and biotic and abiotic stress response–associated genes, as well as genes encoding transcription factors involved in oxygen response. Many of these transcription factors contain the Pro sequence that is a substrate for P4H1. P4H1-catalyzed insertion of Hyp into one or more of these transcription factors may initiate a cascade of responses that include the root hair phenotype observed in plants that overexpress P4H1 (*11*). This leaves open the question as to whether proteins other than cell wall structural proteins may also be direct targets of the P4Hs and thereby candidate regulators of the observed root hair phenotypes. A challenge for the future is to rigorously test the model of Velazquez *et al*. by assessing the substrates for prolyl hydroxylases and the biological role of Hyp in cell wall HRGPs, as well as in regulatory and signaling proteins.

References

1. C. R. Bertozzi, D. Rabuka, in *Essentials of Glycobiology*: *Structural Basis of Glycan Diversity,* A. Varki *et al.*, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2009), chap. 2, pp. 23–36.
2. S. M. Velasquez *et al*., *Science* **332**, 1401 (2011).
3. H. H. Qi *et al*., *Nature* **455**, 421 (2008).
4. W.-C. Hon *et al*., *Nature* **417**, 975 (2002).
5. K. Ohyama, H. Shinohara, M. Ogawa-Ohnishi, Y. Matsubayashi, *Nat. Chem. Biol.* **5**, 578 (2009).
6. G. Pearce, C. A. Ryan, *J. Biol. Chem.* **278**, 30044 (2003).
7. K. L. Gorres, R. T. Raines, *Crit. Rev. Biochem. Mol. Biol.* **45**, 106 (2010).
8. R. Hieta, J. Myllyharju, *J. Biol. Chem.* **277**, 23965 (2002).
9. P. Tiainen, J. Myllyharju, P. Koivunen, *J. Biol. Chem.* **280**, 1142 (2005).
10. E. Shpak, E. Barbar, J. F. Leykam, M. J. Kieliszewski, *J. Biol. Chem.* **276**, 11272 (2001).
11. M. H. Asif, P. K. Trivedi, P. Misra, P. Nath, *Funct. Integr. Genomics* **9**, 525 (2009).
12. M. C. Cannon *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **105**, 2226 (2008).
13. S. Gille, U. Hänsel, M. Ziemann, M. Pauly, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 14699 (2009).
14. J. Egelund *et al*., *Plant Mol. Biol.* **64**, 439 (2007).
15. B. L. Petersen, K. Faber, P. Ulvskov, *Annu. Plant. Rev.* **41**, 305 (2011).

10.1126/science.1208641