Wetting of phase-separated droplets on plant vacuole membranes leads to a competition between tonoplast budding and nanotube formation

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Edited by David A. Weitz, Harvard University, Cambridge, MA, and approved August 4, 2021 (received for review April 27, 2021)

Seeds of dicotyledonous plants store proteins in dedicated membrane-bounded organelles called protein storage vacuoles (PSVs). Formed during seed development through morphological and functional reconfiguration of lytic vacuoles in embryos (M. Feeney et al., Plant Physiol. 177, 241–254 (2018)), PSVs undergo division during the later stages of seed maturation. Here, we study the biophysical mechanism of PSV morphogenesis in vivo, discovering that micrometer-sized liquid droplets containing storage proteins form within the vacuolar lumen through phase separation and wet the tonoplast (vacuolar membrane). We identify distinct tonoplast shapes that arise in response to membrane wetting by droplets and derive a simple theoretical model that conceptualizes these geometries. Conditions of low membrane spontaneous curvature and moderate contact angle (i.e., wettability) favor droplet-induced membrane budding, thereby likely serving to generate multiple, physically separated PSVs in seeds. In contrast, high membrane spontaneous curvature and strong wettability promote an intricate and previously unreported membrane nanotube network that forms at the droplet interface, allowing molecule exchange between droplets and the vacuolar interior. Furthermore, our model predicts that with decreasing wettability, this nanotube structure transitions to a regime with bud and nanotube coexistence, which we confirmed in vitro. As such, we identify intracellular wetting (J. Agudo-Canalejo et al., Nature 591, 142–146 (2021)) as the mechanism underlying PSV morphogenesis and provide evidence suggesting that interconvertible membrane wetting morphologies play a role in the organization of liquid phases in cells.

Author contributions: H.K., N.M., L.F., and R.L.K. designed research; H.K., M.F., J.F.M., and R.L.K. performed research; H.K., J.F.M., and R.L.K. analyzed data; and H.K., A.I.M., L.F., and R.L.K. wrote the paper.

The authors declare no competing interest.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2024109118/-/DCSupplemental.

Published September 2, 2021.
bending of cylindrical nanotubes while assuming they are immersed at the droplet interface, $\alpha\beta$, with an angle equal to the intrinsic contact angle, $\theta_m$ (Fig. 2A and SI Appendix, Extended Theoretical Methods). Such adsorption lowers the interfacial energy. The contact angle $\theta_m$ quantifies the relative interaction strength between $\alpha$, $\beta$, and the membrane.

By minimizing the total energy of the system $E_{\text{total}} = E_{\text{cap}} + E_{\text{tube}}$, we identified three distinct morphological regimes depending on two key parameters: the contact angle $\theta_m$ and the normalized spontaneous curvature $m = (8\kappa m^2 / \Sigma_{\alpha\beta})^{1/2}$ (Fig. 2B). Here, $m$ is the membrane spontaneous curvature, $\kappa$ is the membrane bending rigidity, and $\Sigma_{\alpha\beta}$ denotes the droplet interfacial tension. In regime I, small $m$ values do not favor the formation of membrane nanotubes; instead, excess membrane area results in budding only. For larger $m$, nanotubes form and localize to $\alpha\beta$ interface in two distinct morphologies: either coexisting with membrane buds (regime II, intermediate $m$) or forming as a network of nanotubes exclusively, without buds (regime III, high $m$). We found that, as $\theta_m$ increases, all regime boundaries shift to higher $m$ values (Fig. 2B).

We identified the boundary between regimes I and II to be when the nanotube area, $A_{\text{tube}}$, deviates from zero. To distinguish regimes II and III, we employed a criterion based on the apparent reduced organellar volume being close to a spherical shape with $V_o = (V_{\text{total}})/[(4/3)A_c/4\pi] = 0.99$, with $A_c$ corresponding to the membrane area stored in both spherical caps and $V_{\text{total}}$ accounting for volumes of both interior liquids $\alpha$ and $\beta$. The phenomenon observed is robust. Variations in $\theta_m$ only slightly shift the regime boundary. Hence, droplet-mediated organelle remodeling can be understood as a competition between nanotube and bud formation.

Consistent with our model, we observed three tonoplast morphologies in living embryos (Fig. 1 D–F). However, whether and how droplet and membrane physical parameters change to affect tonoplast shape transformations are not known. While...
regimes I and II have previously been observed in vitro using vacuole-sized vesicles enclosing two polymer liquids (6, 7) (SI Appendix, Extended Experimental Methods). Regime III has not. In this experimental system, increased osmotic pressure raises both \( \Sigma_{\alpha\beta} \) and \( \theta_{\alpha\beta} \) (8). In agreement with our model, we observed regime III shapes that were stable for over 10 h under conditions of low osmotic pressure close to the polymer phase separation point (i.e., characterized by low \( \Sigma_{\alpha\beta} \) and \( \theta_{\alpha\beta} \), Fig. 2C). Meanwhile, under high osmotic pressure, we observed regime II shapes (Fig. 2D). Using time-lapse imaging, we directly confirmed that exposure of regime III vesicles to hyperosmotic stress resulted in regime III to II remodeling (Fig. 2E), as predicted by our model.

Our models rationalize tonoplast remodeling, a main morphological event during PSV formation, using simple theoretical and in vitro parameters. While our models recapitulate key PSV shapes, how PSVs form is still not well characterized and future investigations must address discrepancies between in vivo PSVs and modeled predictions for controlled conditions. For example, we observed that single preexisting vacuoles generate many PSVs, and that tonoplasts are not immediately deformed by storage protein droplets. Multiple PSVs might result from tonoplast ridges that form between adjacent droplets, thereby limiting droplet fusion and causing consecutive rounds of droplet formation and budding. Furthermore, the combination of ongoing storage protein accumulation, water efflux, and decreasing pH likely provides a means of tuning droplet properties, tonoplast charge, and membrane spontaneous curvatures, thereby controlling organellar wetting morphologies. In addition, external factors such as tonoplast–cytoskeleton linkages, the viscoelasticity of the cytosol, and a broad range of organelles including oil bodies might slow and sterically constrain tonoplast deformation substantially, while low droplet interfacial tension and an absence of membrane excess area might prevent membrane deformation altogether. Indeed, the process of *A. thaliana* PSV formation is known to be asynchronous and slow, taking several days (1).

Beyond understanding the functional basis of protein accumulation for crop improvement, our findings promise a means of engineering PSVs, potentially allowing for the development of new sources of high-value proteins (9). We show that, together, droplet and membrane material properties determine whether networks of nanotubes wet droplets or result in droplet-mediated formation of membrane buds. Our data suggest that the key mode of PSV formation is budding: While a bud can reversibly separate two liquid phases and establish distinct intracellular milieus by enclosing each within physically discrete membranes, wetting nanotube networks provide a structure allowing for molecule exchange between both liquid phases (Fig. 2 B and C). This work demonstrates both how droplets provide a liquid structure for assembling competing membrane shapes, as well as an example of how membrane wetting organizes liquids in cells.

**Materials and Methods**

All data, materials, and equations needed to evaluate the conclusions in the paper are provided in the paper. Additional data related to this manuscript may be requested from the authors.

**Data Availability.** All study data are included in the article and/or supporting information.

**ACKNOWLEDGMENTS.** We thank Kengo Watanabe and Hidenori Ichijo (Graduate School of Pharmaceutical Sciences, The University of Tokyo [UT]) for providing access to the osmometer. R.L.K. and H.K. thank colleagues at Max Planck Institute of Colloids and Interfaces for discussion. R.L.K. thanks the Warwick Institute for Advanced Studies for an International Visiting Fellowship, Cheiko Saito (UT) for inspiration, and Christian Schmitz-Linneweber, Cornelia Stock, and Thomas Korte (Humboldt University of Berlin) for support. N.M. was supported by the Exploratory Research for Advanced Technology via the Japan Science and Technology Agency (JPMJER1702). L.F. and J.F.M. were supported by a grant from the Leverhulme Trust (RPG-20-013). A.I.M. was supported by Japan Society for the Promotion of Science KAKENHI Grant JP21K15083. H.K. was supported by Engineering and Physical Sciences Research Council (EP/P007139/1 and EP/J017566/1).

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