Snowpack in the Colorado River basin is a solar-radiation shield that suppresses water loss through evaporation. This protection decreases as local temperatures rise.

warm versus cold years, they square observational estimates of sensitivity with substantially lower estimates from hydrologic model sensitivity studies.

The E_0 used by Milly and Dunne is more informative than approaches based only on temperature; their earlier work argues that radiation-based measures capture ET changes in "water-rich" areas better than do other methods (*12*). However, it is reasonable to ask whether the parameterization used for E_0 in the new study is the best measure for the mixed energy- and water-limited hydroclimates across the UCRB. Also, does the choice of solar radiation (and to some extent, temperature) as the driver of E_0 force the finding that albedo plays the dominant role in determining streamflow from their hydrology model?

The 9.3% loss of streamflow per degree Celsius of warming cannot reconcile all available data and model dynamics; global and regional climate models and hydrologic models also include the albedo effect yet show differing sensitivities. Other missing pieces of the puzzle include the effects of dust on snow, the direct effects of increasing CO_2 concentrations on trends in the longwave radiation balance and on vegetation's water-use efficiency, and land-cover changes from wildfires and insect outbreaks.

The year 2020 is a momentous one for CR water policy. The interim interstate agreement on sharing water-shortage impacts will be renegotiated this year. The new, more stringent Lower Basin Drought Contingency Plan will mandate that water deliveries to states in the lower basin be reduced—a first, and unthinkable a generation ago. These adaptation strategies are difficult in a single snowmelt–driven basin in a wealthy country. How to approach such problems in similar basins worldwide is an open question. ■

GRAPHIC: A. KITTERMAN/*SCIENCE*

REFERENCES AND NOTES

1. T. James, A. Evans, E. Madly, C. Kelly, *The Economic Importance of the Colorado River to the Basin Region* (Seidman Research Institute, 2014).
2. J. J. Barsugli *et al.*, *Eos* 10.1029/2019EO117173 (2019).
3. P. C. D. Milly, K. A. Dunne, *Science* **367**, 1252 (2020).
4. H.-O. Pörtner *et al.*, *IPCC Special Report on the Ocean and Cryosphere in a Changing Climate* (Intergovernmental Panel on Climate Change, 2019).
5. J. A. Vano *et al.*, *Bull. Am. Meteorol. Soc.* **95**, 59 (2014).
6. M. T. Hobbins *et al.*, *Geophys. Res. Lett.* **35**, L12403 (2008).
7. J. Sheffield *et al.*, *Nature* **491**, 435 (2012).
8. M. T. Hobbins *et al.*, *Geophys. Res. Lett.* **31**, L13503 (2004).
9. M. L. Roderick *et al.*, *Geogr. Compass* **3**, 761 (2009).
10. F. Lehner *et al.*, *Nat. Clim. Chang.* **9**, 926 (2019).
11. M. P. Hoerling *et al.*, *J. Clim.* **32**, 8181 (2019).
12. P. C. D. Milly, K. A. Dunne, *Nat. Clim. Chang.* **6**, 946 (2016).

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MOLECULAR BIOLOGY

Liquid droplets in the skin

Creating enough glue to protect the body may require phase separation in skin cells

By **Arpan Rai** *and* **Lucas Pelkmans**

Liquid-liquid phase separation (LLPS), the unmixing of inhomogeneous fluids into two or more phases, is emerging as a paradigm for the formation of a myriad of membraneless compartments inside cells (*1*, *2*). This type of spatial organization, in contrast to membrane-bound compartmentalization, has long lacked unifying principles. However, the physiological relevance of compartmentalization through LLPS inside cells is still poorly understood and often speculative. Additionally, regulatory mechanisms through which cells control and exploit LLPS are still emerging. On page 1210 of this issue, Garcia Quiroz *et al.* (*3*) show that keratohyalin granules (KGs) that are formed during epidermal differentiation in the skin are pH-sensitive liquid-like protein condensates. Formation of KGs may be physiologically important because mutations that cause defects in this process are associated with the common skin barrier defect ichthyosis vulgaris.

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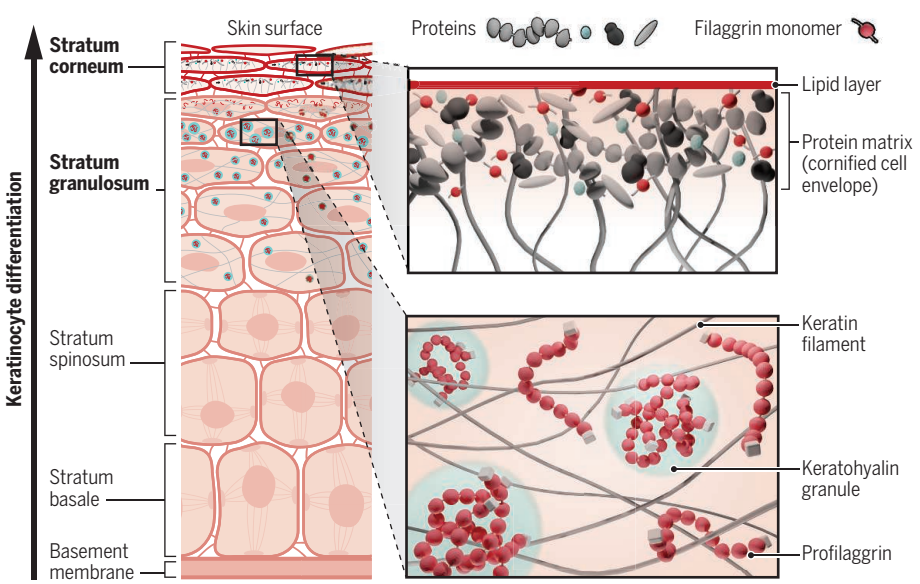
The most external part of skin, the epidermis, is composed of keratinocytes, whose morphological appearance changes as they differentiate, resulting in various layers. In the stratum granulosum (granular layer), which sits just below the stratum corneum (cornified layer) in which the keratinocytes expel their nuclei and form a continuous water-impermeable protective zone, keratinocytes transiently contain KGs. These appear as electron-dense, protein-rich structures that lack a delimiting membrane (*4*). Are KGs physiologically relevant? A core component of KGs is the protein profilaggrin, which is cleaved into individual repeats (filaggrin monomers) when the stratum corneum forms. In this layer, filaggrin monomers function as part of the "glue" that forms the impermeable barrier of the skin. Mutations that result in a smaller number of filaggrin repeats lead to disappearance of KGs. The in-

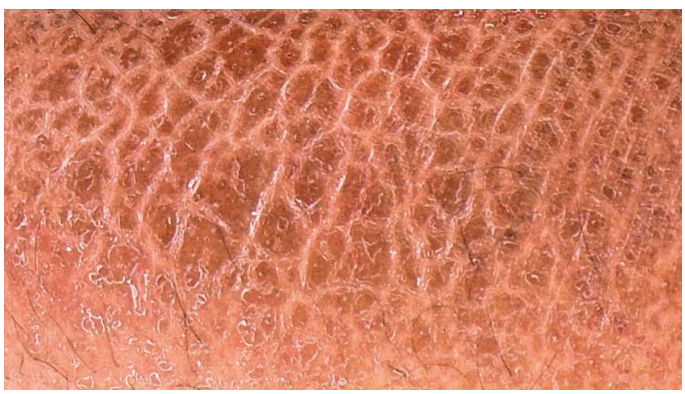
Liquid-liquid phase separation in the skin

Keratohyalin granules (KGs), formed by liquid-liquid phase separation of profilaggrin, interact with keratin filaments to organize the cytoplasm of keratinocytes during differentiation. During transition of keratinocytes from the stratum granulosum (granular layer) to the stratum corneum (cornified layer), KGs dissolve and profilaggrin is processed into monomers, which together with other proteins, contribute to the formation of a solid intracellular protein matrix.



ability to form KGs limits the expression of filaggrin monomers (*5*), which results in improper barrier formation. This is where LLPS may play a crucial role.

Garcia Quiroz *et al.* show that profilaggrin can undergo LLPS inside cultured keratinocytes and that this phenomenon depends on the number of filaggrin repeats. From synthetic systems, it is known that the concentration at which multivalent repeats undergo LLPS, as well as the composition of condensates formed, is sensitive to the valency and stoichiometry of the repeats (*6*). Because skin barrier disorder–associated profilaggrin mutations result in variable numbers of filaggrin repeats, these may reflect a disease-causing manifestation of altered multivalency of a phase-separating protein, affecting the composition and dynamics of the cellular condensates they form. Using live imaging of engineered phase separation sensors transduced in embryonic mouse skin epithelium, Garcia Quiroz *et al.* show that endogenous KGs behave like condensates, increasing in number and stiffness as keratinocytes differentiate and progress through the stratum granulosum. This may be mediated by the KGs undergoing a liquid–to–gel-like transition during differentiation, as well as by the dense network of intermediate filaments in which KGs grow and with which they interact (see the figure). Macromolecular crowding can affect phase separation properties of proteins both in vitro and inside cultured cells (*7*). Moreover, as shown in a nonbiological system, the density and elasticity of polymer networks can affect the protein solubility threshold at which LLPS occurs, as well as the size and nucleation of condensates (*8*).



The skin condition ichthyosis vulgaris is likely caused by defective liquid-liquid phase separation of profilaggrin and hence improper barrier formation in the skin.

Although aberrant liquid–to–gel-like transitions have been speculated to underly the appearance of protein aggregates in neurodegenerative diseases (*2*), the study of Garcia Quiroz *et al.* indicates that in certain contexts, such transitions are physiologically important—namely, to form a protective layer in the skin. Also during the formation of gel-like aggregates in neurodegenerative diseases, the cytoskeleton (*9*) might play a role in modulating their stiffness. In the future, it will be important to understand how condensates and cytoskeletal networks affect each other to structure the interior of the cell and how this synergy is perturbed in disease.

Garcia Quiroz *et al.* also reveal that both endogenous KGs in the skin and profilaggrin condensates in cultured cells sense pH changes, responding to the drop in pH that occurs when cells approach the stratum corneum. The lower pH triggers KG dissolution, leading to increased amounts of profilaggrin in the cytoplasm. Such environmental sensing has been shown for other phase-separating proteins, such as Sup35 and polyadenylate-binding protein (Pab1) in yeast (*10*). KG dissolution may also be aided by changes in intracellular Ca^{2+} concentrations that occur during epidermal differentiation (*11*), because keratinocytes express many Ca^{2+} binding proteins, including profilaggrin. Furthermore, although not addressed by the authors, profilaggrin becomes extensively phosphorylated in the granular layer, and subsequently dephosphorylated before being processed into monomers (*5*). Cycles of phosphorylation and dephosphorylation have been shown to regulate multiple condensates (*12*)—for instance, during stress recovery (*13*) and progression through mitosis (*14*). This provides another attractive, actively controlled mechanism by which keratinocytes could modify the critical concentration at which profilaggrin undergoes LLPS.

The processing of profilaggrin into individual filaggrin monomers is mediated by proteases, which have been shown in synthetic in vitro systems to rapidly change the valency of repeats of engineered proteins, thereby triggering the dissolution of condensates (*15*). It thus seems plausible that a multimodal mechanism is in place to ensure that condensation and subsequent dissolution of KGs in keratinocytes is robust and precisely timed during epidermal differentiation.

But why undergo LLPS to produce KGs, when they disappear and profilaggrin is cleaved into monomers in the layer above? Many functions have been proposed for condensates, including storage, modulation of signaling, environmental stress sensing, force generation, and noise buffering (*1*, *2*). Garcia Quiroz *et al.* posit that KGs, along with the keratin network, function to physically deform the nucleus, prior to enucleation. It is an attractive hypothesis, considering that the stiffness of KGs and the density of the keratin network increase during epidermal differentiation. However, it is also possible that KGs act as a storage depot for profilaggrin, protecting it from proteolytic processing. Exclusion of proteases from KGs would prevent premature processing of profilaggrin. Moreover, there are high amounts of the amino acid histidine in profilaggrin, which is metabolically converted to organic acids upon proteolytic cleavage in the stratum corneum, contributing to skin acidification. Therefore, premature processing of profilaggrin could strongly affect the intracellular milieu when it is in high abundance. Concentrating profilaggrin through LLPS and formation of KGs may thus protect the cell from possible deleterious effects of premature acidification.

Future studies are required to address whether sequestering profilaggrin or other epidermal regulators in condensates, which might otherwise have toxic effects at high cellular concentrations, allows cells to build up enough material and temporally regulate their release. The released proteins may then transition into a continuous, irreversible solid protein matrix of the stratum corneum by enzymes that introduce covalent cross-links. Multicellular systems that recapitulate the three-dimensional structure of tissues will become increasingly important to explore how LLPS is exploited and regulated in cells within the context of a tissue. By generating new properties in form and function at the supramolecular scale, LLPS may provide key insights into the mechanisms by which biological scales are connected, and how this goes wrong in disease. ■

REFERENCES AND NOTES

1. S. F. Banani, H. O. Lee, A. A. Hyman, M. K. Rosen, *Nat. Rev. Mol. Cell Biol.* **18**, 285 (2017).
2. Y. Shin, C. P. Brangwynne, *Science* **357**, eaaf4382 (2017).
3. F. G. Quiroz *et al.*, *Science* **367**, eaax9554 (2020).
4. K. A. Holbrook, *J. Invest. Dermatol.* **92**, S84 (1989).
5. A. Sandilands, C. Sutherland, A. D. Irvine, W. H. I. McLean, *J. Cell Sci.* **122**, 1285 (2009).
6. S. F. Banani *et al.*, *Cell* **166**, 651 (2016).
7. M. Delarue *et al.*, *Cell* **174**, 338 (2018).
8. R. W. Style *et al.*, *Phys. Rev. X* **8**, 011028 (2018).
9. J. Dubey, N. Ratnakaran, S. P. Koushika, *Front. Cell. Neurosci.* **9**, 343 (2015).
10. H. Yoo, C. Triandafillou, D. A. Drummond, *J. Biol. Chem.* **294**, 7151 (2019).
11. P. Elias, S. Ahn, B. Brown, D. Crumrine, K. R. Feingold, *J. Invest. Dermatol.* **119**, 1269 (2002).
12. J. Söding, D. Zwicker, S. Sohrabi-Jahromi, M. Boehning, J. Kirschbaum, *Trends Cell Biol.* **30**, 4 (2020).
13. F. Wippich *et al.*, *Cell* **152**, 791 (2013).
14. A. K. Rai, J.-X. Chen, M. Selbach, L. Pelkmans, *Nature* **559**, 211 (2018).
15. B. S. Schuster *et al.*, *Nat. Commun.* **9**, 2985 (2018).

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