LETTER

Mucus supramolecular topology: An elusive riddle

The supramolecular conformation that keeps mucins interconnected in the mucus gel matrix is still uncertain. In the granule, a Ca-bonded matrix keeps mucus condensed. Upon exocytosis, Na/Ca exchange removes Ca bonds, triggering characteristic polymer gel phase transition and massive swelling (1). Failure to remove Ca by increased extracellular Ca inhibits mucus hydration (2). Transepithelial movement of $^{-}HCO_3$ is defective in cystic fibrosis, and the demonstration that $^{-}HCO_3$ can modulate mucus swelling (3) suggests that deficient $^{-}HCO_3$ Ca buffering might be at the source of cystic fibrosis mucus pathology.

The model proposed in PNAS (4) explains how, during storage, mucins are folded, forming densely Ca-bonded arrays. Following Ca removal during exocytosis, mucin arrays can drastically expand as mucus swells. EM of glutaraldehyde-fixed mucins shows images that resemble Adrian Allen's "windmill" model of crosslinked mucins. Ambort et al. (4) proposed that the disulfide-bonded junctions in the windmill core would function as hinges allowing the collapse of glycosylated mucin blocks during storage, or their unfolding upon swelling. Unfolding would form a 2D netlike structure of interconnected polygons that can expand over several square microns. Accordingly, EM sections of goblet-cell granules show corresponding interconnected polygonal images. However, similar images can be found in EM sections of a broad variety of chemically fixed granules. A quick PubMed review of the literature shows that EM renders analogous pictures in secretory granules of snake venom secretory cells, snail and hydra granules, and granules from prolactin cells, pancreatic cells, white blood cells, thyroid cells, parathyroid cells, platelets, mast cells, and von Willebrand factor of Weibel–Palade bodies of endothelial cells, among others. This remarkable similarity depicting irregular arrays of interconnected polygon-like structures in granules containing very different biopolymer networks is surprising. The common denominator in this case is the use of fixative agents, suggesting that interconnections might portray chemical crosslinking rather than the actual supramolecular conformation of the granule polymer matrix. In fact, EM images from glutaraldehyde-fixed colonic goblet cells render an interconnected netlike structure of the granular matrix. However, cryofixed images of these cells fail to report interconnected polygonal structures (5). The presence of nematic liquid crystalline lattices in native nonfixed mucus reveals the existence of ordered domains (1). However, detailed topological features of how mucins are folded inside the granule still remain uncertain.

The model of Ambort et al. (4) further disagrees with video recordings of mucus swelling kinetics upon release from goblet cells and with images of microscopic exocytosed mucus. In fact, the proposed model (4) implies a planar highly unisotropic expansion of the matrix that, as pointed out by the authors, could explicate the typical multilayer mucus coating on the guts surface. However, available results show that, following release from the cell, mucus undergoes characteristic 3D isotropic swelling, resulting in the formation of spherical microgels like those described in previous reports (1–3) and shown in another recent study published in *The Journal of Experimental Medicine* by Hansson's group.* Disagreement with physical dynamics of the mucus gel, and built-in uncertainties of chemically fixed EM images, fail to provide a solid foundation to this otherwise novel supramolecular mucus model.

Pedro Verdugo[1]
Departments of Bioengineering and Internal Medicine, University of Washington, Seattle, WA 98250

1. Verdugo P (2012) Supramolecular dynamics of mucus. *Cold Spring Harb Perspect Med*, 10.11.01/cshperspect.a009597.
2. Verdugo P, Aitken ML, Langley L, Villalon MJ (1987) Molecular mechanism of product storage and release in mucin secretion. II. The role of extracellular Ca^{++}. *Biorheology* 24:625–633.
3. Chen EY, Yang N, Quinton PM, Chin WC (2010) A new role for bicarbonate in mucus formation. *Am J Physiol Lung Cell Mol Physiol* 299:L542–L549.
4. Ambort D, et al. (2012) Calcium and pH-dependent packing and release of the gel-forming MUC2 mucin. *Proc Natl Acad Sci USA* 109:5645–5650.
5. Nagata T (2009) Applications of high voltage electron microscopy to thick biological specimens. *Ann Microscopy* 9:4–40.

Author contributions: P.V. wrote the paper.

The author declares no conflict of interest.

*Gustafsson JK, et al. (2012) Bicarbonate and functional CFTR channel are required for proper mucin secretion and link cystic fibrosis with its mucus phenotype. *J Exp Med*, 10.1084/jem.20120562.

[1]E-mail: verdugo@u.washington.edu