LETTER

Reply to Verdugo: Mucins form highly organized supramolecular structures

The model of mucins as enormously large linear polymers with limited organization has dominated the mucus field. Twenty-five years ago, Verdugo et al. (1) suggested that the mucins are stored as such in the regulated secretory pathway of goblet cells and packed by calcium ions shielding the negative charges (sialic acid and sulfate groups) of the highly glycosylated mucin domains (1, 2). That Ca^{2+} and low pH are necessary for packing mucins is not controversial, as this is the case for all cell types with regulated secretory machinery. Typical for such granulae, including goblet cells, as shown by Tetsuji et al. (3) and referred to by Verdugo (2), are packed electron-dense protein cores often without discernible structure. Unpacking of such granulae involves bicarbonate-mediated removal of Ca^{2+}. When studying goblet cells during MUC2 unpacking, we observed the ringlike structures (4) that Verdugo suggests to be crosslinking artifacts (2). Fixation for EM might cause artifacts, but these rings are in line with the findings of other experiments, supporting our model.

Our view of MUC2 packing is largely based on the recent understanding of von Willebrand factor (vWF), a molecule that evolved from the mucins and has a to mucins similar domain organization in its N-terminal 1,200 aa (5). vWF is packed as linear spirals, and glutaraldehyde cross-linking of endothelial cells with Weibel–Palade bodies shows EM with tubular structures (5) and not interconnected polygonal structures as stated by Verdugo (2). Release of vWF is by a slow unwinding of the linear polymer as it is pulled out from the vesicle by the vascular blood flow. This is in contrast to the mucin release in which the packed mucin is expanded 1,000-fold in seconds. This process requires a well-organized packing in the storage granulae such as the one suggested by our model with N-terminal trimers packed with the help of Ca^{2+} ions into concatenated rings (4). The Verdugo model (2) with randomly packed mucins is not compatible with such a fast release without entanglements.

The released MUC2 mucin will produce relatively flat sheets that we suggest are staggered on top of each other (4). However, we have claimed this is the case for only the inner mucus layer of colon, in which this suits its function to separate the intestinal bacteria from the epithelial cells (6). Our observation that this stratified layer can be converted into the outer mucus layer by a three- to fivefold 3D swelling caused by MUC2 mucin proteolysis shows that a mucin can be transformed into other mucus forms (6). The outer colon mucus layer is more typical for mucus and shows that our model is compatible with 3D swelling as referred to by Verdugo (2). The MUC2 mucin of the small intestine does not form stratified mucus, and we now know this is also a result of specific proteolysis in the MUC2 mucin. It should, however, be pointed out that it is possible that the other human gel-forming mucins (MUC5AC, MUC5B, and MUC6) do not follow our MUC2 model.

Daniel Ambort and Gunnar C. Hansson[1]
Department of Medical Biochemistry, University of Gothenburg, 405 30 Gothenburg, Sweden

1. Verdugo P, Deyrup-Olsen I, Aitken M, Villalon M, Johnson D (1987) Molecular mechanism of mucin secretion: I. The role of intragranular charge shielding. *J Dent Res* 66:506–508.
2. Verdugo P (2012) Mucus supramolecular topology: An elusive riddle. *Proc Natl Acad Sci USA* 109:E2956.
3. Tetsuji N (2009) Applications of high voltage electron microscopy to thick biological specimens. *Ann Microscopy* 9:4–40.
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. Springer TA (2011) Biology and physics of von Willebrand factor concatamers. *J Thromb Haemost* 9(suppl 1):130–143.
6. Johansson MEV, et al. (2008) The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci USA* 105:15064–15069.

Author contributions: D.A. and G.C.H. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. E-mail: gunnar.hansson@medkem.gu.se.