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Research reveals details of how salivary glands collectively produce constellation of proteins found in saliva

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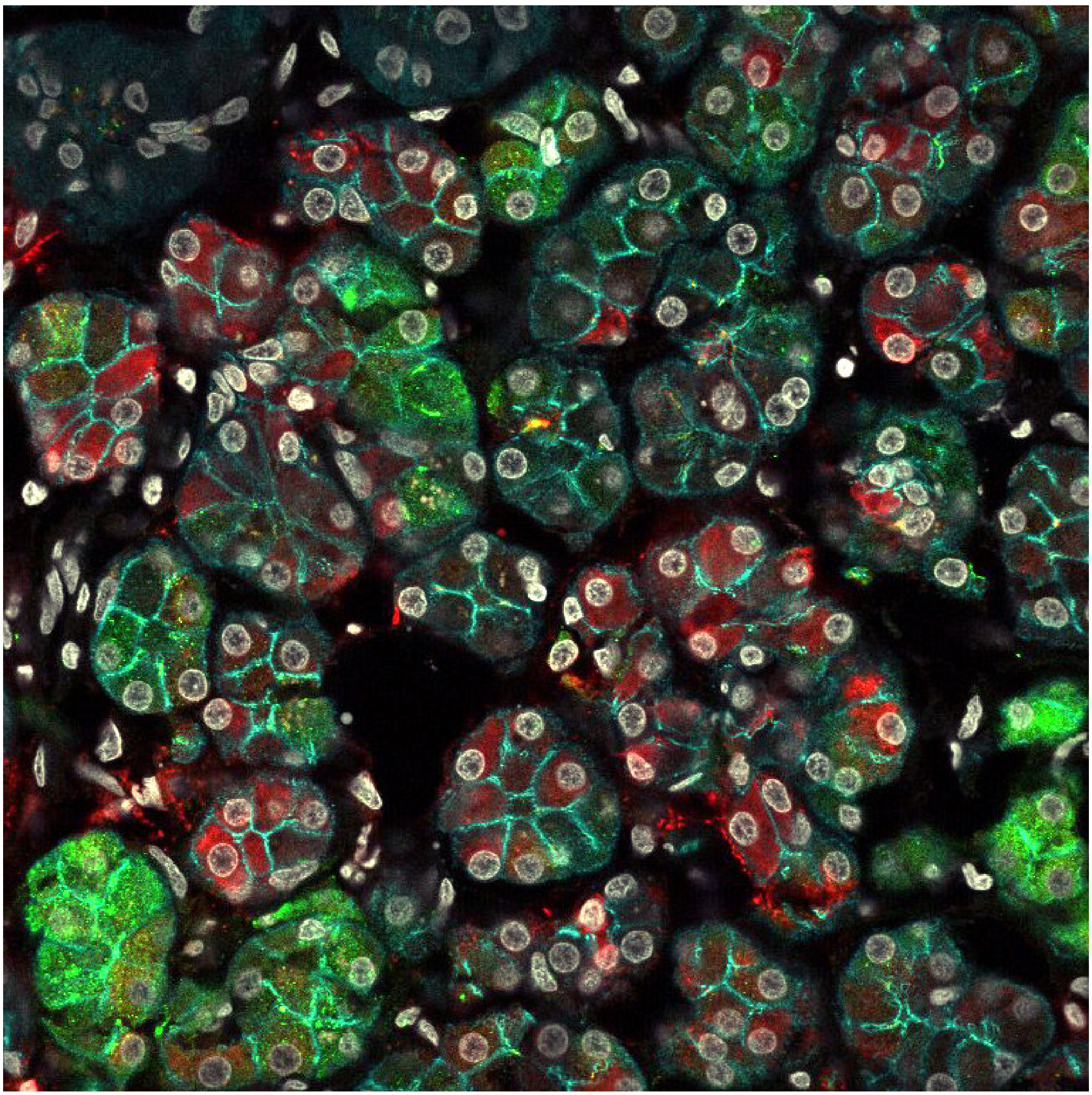

An immunofluorescence microscopy image of cells in a human submandibular gland. The image reveals that secretory acinar cells, which were all thought to be the same, are actually much more diverse, as shown by the different cells in the same gland expressing different proteins. A green stain indicates the presence of mucin 7, and a red stain indicates the presence of amylase. Nuclei of cells are in grey, and the epithelial membrane in cyan. Credit: Alison May

In the TV series, "How It's Made," viewers often discover that common objects like pencils or rubber bands are quite complicated to make. The show walks people through complex production processes that lie behind familiar items.

A new paper in the journal *Cell Reports* does the same for saliva.

The study, which will be published on Nov. 17, breaks down, in detail, where the multitude of proteins floating in our saliva originate.

The research traces these vital proteins back to their source, showing which proteins are produced by each of the three major types of human salivary glands, and showing how individual cells within a single gland can secrete different proteins. The project also identifies proteins in the mouth that seem to be coming from outside of salivary glands, from places such as epithelial tissues or blood plasma.

"Saliva is important for tasting, for digesting, for swallowing, for defending us from the pathogens that we are constantly inhaling and consuming. The proteins in our mouth form an army, if you will, that's working constantly to protect us. Before this, scientists had an idea of the proteins that are found in the mouth, but we didn't have a complete picture of where they were coming from. We're addressing this gap," says Omer Gokcumen, Ph.D., associate professor of biological sciences in the University at Buffalo College of Arts and Sciences.

"From a biomedical perspective, our research opens the door for further studies into the functions of saliva and salivary glands, and the use of saliva as a diagnostic fluid. Our study takes a snapshot of how healthy salivary glands should function. Deviations from this healthy expectation can indicate disease," says Stefan Ruhl, Ph.D., DDS, professor of oral biology in the UB School of Dental Medicine.

The study's first author is Marie Saitou, Ph.D., a tenure-track researcher in biosciences at Norwegian University of Life Sciences, and a former postdoctoral researcher at the University of Chicago and UB. Saitou, Gokcumen and Ruhl led the study with Sarah Knox, Ph.D., associate professor of cell and tissue biology in the University of California, San Francisco (UCSF) School of Dentistry.

The biological factories that churn out our saliva

To explain how our bodies make saliva, the scientists first sought to understand which proteins are produced by each major type of salivary gland—the parotid, submandibular and sublingual glands (humans have a pair of each).

To do this, the team used a method called transcriptomics to measure gene activity in each kind of gland. Gene activity provides insight into protein production, because each gene provides instructions for making a specific protein.

This endeavor enabled the scientists to understand the proteins that each gland generates, and how the glands differ from one another in terms of what they produce.

For instance, the study finds that the parotid and submandibular glands create a lot of salivary amylase, an enzyme that helps to digest starch, while the sublingual gland makes almost none. Meanwhile, the sublingual gland produces relatively large quantities of certain GalNAc transferases, a family of enzymes that's important in initiating a process called O-glycosylation that attaches a sugar to certain salivary mucin proteins. These are just a couple of examples.

"We show how the actions of different glands collectively help to produce a complex bodily fluid—our saliva," Saitou says.

"Our work reveals that even a gland type itself is not homogenous: the saliva-producing acinar cells, which were once thought to produce the same proteins, and thus be the same cells, actually synthesize distinct saliva proteins, thus indicating a new level of cellular diversity," Knox says.

Gokcumen says the research is one step toward understanding the immense complexity of saliva. Beyond parsing out the origins of proteins made by salivary glands, the team also concluded that some proteins drifting in saliva likely don't originate from salivary glands, and that some important proteins that help to regulate gene expression are predominantly active in salivary glands, but not in a litany of other tissues.

"Salivary proteins are a gateway to our body," Gokcumen says. "When they do not function properly, we suffer. Our work brings us one step closer to understanding their complex origins and the intricate interplay between them."

"Long wished-for diagnostic applications of saliva for monitoring systemic well-being and disease will need to measure quantitative differences of biomarkers in saliva," Ruhl says. "One obstacle always hampering progress in this arena was that we did not know exactly which proteins were intrinsically produced by the salivary glands, and which proteins diffused into saliva from surrounding tissue leakage. Also, we were lacking a reliable baseline, a standard, if you will, that tells us what are normal and healthy values for the protein components in saliva. Our paper helps resolve these conflicts, providing information that I expect will propel salivary diagnostic applications forward."

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