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# Sweet recipe for cellular closeness

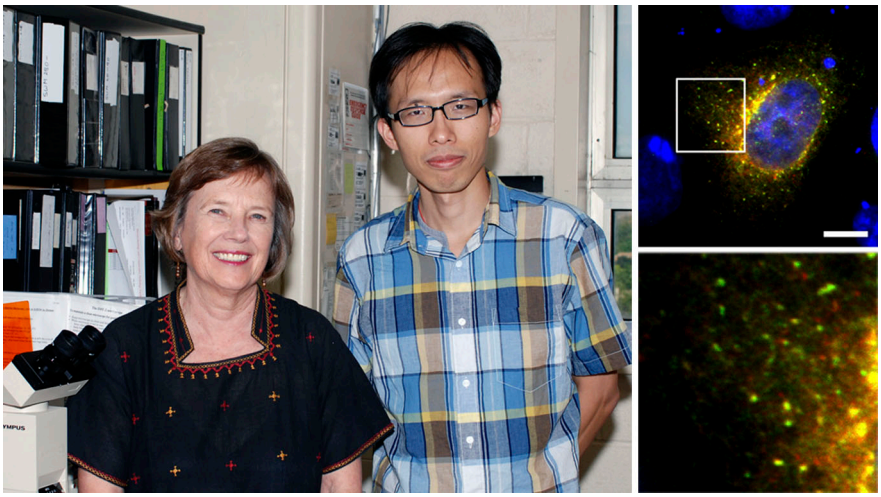
Protein halts modification of glycoproteins, encouraging germ cells to stay tight.

Testicular germ cells are clingy, adhering to the cells that nurture them. Huang and Stanley (1) reveal a protein whose activity promotes this togetherness by making glycoproteins on the germ cells' surface stickier.

Many proteins sport carbohydrate attachments called N-glycans (2). Cells affix and modify N-glycans as a glycoprotein wends its way through the secretory pathway (3). How a protein folds, where it travels in the cell, how soluble it is, whether it catches the attention of the immune system, and other characteristics can depend on its N-glycan complement. N-glycans fall into three main categories. High mannose varieties contain multiple copies of the sugar mannose. The hybrid and complex forms, by contrast, are synthesized from the high mannose varieties and contain additional sugars. Previously, Pam Stanley's lab found that a mutation in cells called Lec1 prevented them from converting high mannose N-glycans into the complex and hybrid forms (4).

Now, Huang and Stanley have uncovered a mouse protein that blocks the conversion reaction. The pair started by scanning genome sequences for previously unrecognized glycosyltransferases, or glycan-synthesizing enzymes. They identified a candidate that unexpectedly turned out to be a glycosyltransferase inhibitor. To determine if the protein altered N-glycans, the researchers put it through a standard test: some of the plant proteins known as lectins—one example is the notorious toxin ricin—latch onto certain glycans and kill the cells carrying them. If the cells survive, the glycans have changed. Like Lec1 mutant cells, Chinese hamster ovary cells engineered to make the newly discovered inhibitor protein were unfazed by a lectin that destroys cells carrying hybrid or complex N-glycans.

## Focal Point



While looking for enzymes that help make N-glycans, Pam Stanley (left) and Hung-Hsiang Huang stumbled on a protein, GnT1IP, that prevents cells from revamping their high mannose N-glycans. These stickier glycans could help fasten testicular germ cells to their supporting cells. At ER exit sites, GnT1IP (red) cozies up to GlcNAcT-I (green), the enzyme it inhibits.

The researchers tracked the protein, which they dubbed GnT1IP, to its cellular home. GnT1IP settles in the endoplasmic reticulum, the cis side of the Golgi, and the ER-Golgi intermediate compartment that relays cargoes between the two. That puts the protein in a prime location to tweak N-glycan structure. But the protein doesn't act directly on glycans, the team found. Instead, it hooks onto and inactivates GlcNAcT-I, the enzyme that starts the remodeling of high mannose glycans into complex or hybrid ones.

> "Our model is that glycoproteins with high mannose glycans are better able to associate with Sertoli cells."

Together, these results show that GnT1IP causes glycoproteins to maintain their simpler, high mannose N-glycans. A clue to the importance of this ability comes from GnT1IP's restricted distribution in the body. Its gene seems to be active only in the testes, particularly in the germ cells that are the source of sperm. Sertoli cells that hug and coddle the germ cells express little if any GnT1IP. "Germ cells must be nursed by Sertoli cells throughout spermatogenesis," says Stanley. The high mannose N-glycans might make proteins on the surface of a germ cell stickier. "Our model is that glycoproteins with high mannose glycans are better able to associate with Sertoli cells," says Stanley. The researchers tested the model using Chinese hamster ovary cells engineered to manufacture GnT1IP. The modified cells got a better grip on Sertoli cells than did controls.

"GnT1IP's very restricted and regulated expression in testicular germ cells suggests that it has an important role in spermatogenesis," says Stanley. That means defective versions of GnT1IP might cause some cases of infertility—a possibility the researchers would like to investigate. Another potential practical benefit involves antibodies used to treat cancer and autoimmune diseases. These proteins are more potent if they carry high mannose glycans. The findings might lead to new ways to engineer the N-glycans so that the antibodies pack more punch.

1. Huang, H.-H., and P. Stanley. 2010. *J. Cell Biol.* doi:10.1083/jcb.201004102.
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3. Stanley, P., et al. 2009. Chapter 8. N-glycans. *In* Essentials of Glycobiology. A. Varki, et al., editors.
4. Chen, W., and P. Stanley. 2003. *Glycobiology.* 13:43–50.