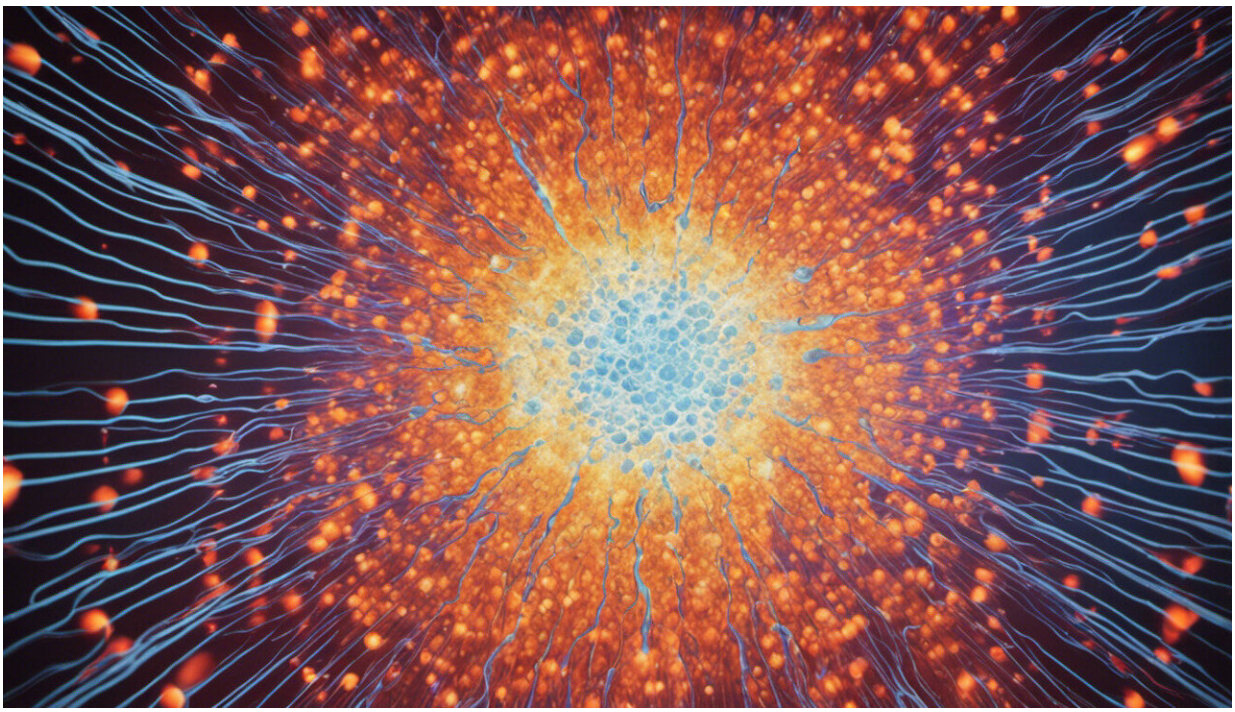
Large-scale screen reveals how numerous signaling pathways intersect at the cell's primary protein-processing center

April 24 2013



Credit: AI-generated image (disclaimer)

Many proteins undergo extensive modification after being synthesized, particularly those that are secreted or embedded in the cell membrane. This is achieved within the Golgi apparatus (see image), a cellular organelle consisting of multiple membrane-bound compartments known

as cisternae. Each of these contains specific sets of protein-modifying enzymes, which sequentially modify their targets. For example, many proteins undergo glycosylation, which entails the stepwise addition of complex sugar molecules.

Golgi function depends heavily on proper organization, particularly in mammalian cells. In an ambitious study, a research team led by Frederic Bard of the A*STAR Institute of Molecular and Cell Biology has identified proteins that maintain this organelle's structure and function. Many critical cellular functions are managed by signaling enzymes that either add or remove phosphate chemical groups from target proteins, known respectively as kinases and phosphatases. Bard and co-workers focused on a set of 948 proteins encompassing most of these enzymes.

The researchers used a technique called RNA interference to specifically reduce production of each protein in cultured cells, and then applied a sophisticated imaging strategy to determine the impact on different subsets of Golgi cisternae. A series of pilot experiments using treatments known to affect Golgi function enabled them to 'train' their imaging software to recognize the physiological hallmarks associated with different disruptions. In parallel, Bard and co-workers applied a targeted fluorescent labeling strategy to 'color code' the various Golgi subcompartments, allowing them to determine which of these were specifically affected in each experiment.

Using the trained imaging algorithm, the researchers identified 159 signaling factors that apparently contribute to Golgi organization and structure. Many of these were directly linked to critical Golgi functions, such as the dynamic behavior of cisternal membranes or the trafficking system that physically shuttles molecules between cisternae. Several of the targets identified specifically transmit signals in response to extracellular cues, indicating that Golgi organization may be greatly affected by the environment outside of the cell.

Importantly, many of these signaling factors exert a particularly strong influence on glycosylation patterns. "The sheer complexity and diversity of glyco-phenotypes arising from signaling-gene depletion were very surprising," says Bard. Given that both signaling pathways and protein glycosylation are highly prone to disruption in cancerous cells, these data suggest that the Golgi could be an important nexus for some tumorigenic processes. Bard will explore this possibility in future work. "We plan to decipher the specific cascades of glycosylation regulation that are frequently activated in tumor cells," he says.

More information: Chia, J., Goh, G., Racine, V., Ng, S., Kumar, P. & Bard, F. RNAi screening reveals a large signaling network controlling the Golgi apparatus in human cells. *Molecular Systems Biology* 8, 629 (2012). www.nature.com/msb/journal/v8/ … /full/msb201259.html

Provided by Agency for Science, Technology and Research (A*STAR), Singapore