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Jim's View: Is the Golgi stack a phase-separated liquid crystal?

We are currently witnessing a revolution in how we think about the compartmentalization of cells. In the 1950s and 1960s we learned that lipid bilayer-based membranes create containers (organelles) within the cytoplasm, each containing a unique set of proteins enabling functional specialization. We are now learning that functionally specialized domains – but without walls of membrane – can form entirely by self-assembly within the cell. These liquid-like "membrane-less organelles" can potentially come and go on demand (which membrane-bound organelles can never do) based on a liquid-liquid phase separations from the rest of the cytoplasm [1]. In many cases phase separation results from intrinsically disordered proteins which can contain specific RNA binding domains [2,3].

The nucleolus [4] is an important example. Nucleoli were recognized by early cytologists as a conspicuous feature within the nucleus, and molecular biologists discovered that they contain and transcribe the genes for ribosomal RNA, process the RNA by various cleavages and covalent modifications, and then combine them with proteins to assemble ribosome subunits. These steps are spatially organized to take place within sequentially encountered sub-compartments with the newly manufactured cargo (rRNAs) passing from one to another, maturing step-by-step until the ribosomal subunits are ready for export to the cytoplasm [4].

It is remarkable that such a well-organized complex structure can come and go. For example, when rRNA transcription is blocked, or certain drugs are added, the nucleoli grossly re-organize or even disassemble. They re-form when conditions are normalized. Physiologically, nucleoli

disassemble in mitosis and re-assemble in daughter cells at the end of cell division. Consistent with this, nucleoli behave overall like liquid droplets, breaking up under pressure into smaller droplets that then fuse back together, and their constituents freely diffuse within them [5]. More recently, in a bold and revealing series of experiments, Brangwynne and colleagues [6] have found that key elements of the layered spherical "droplet within a droplet" liquid organization of nucleoli form spontaneously by liquid-liquid phase separation when constituent RNAs and proteins are mixed in the test tube.

The underlying purpose, sub-compartmental organization, and exceptional plasticity of the nucleolus remind me of the Golgi apparatus [7] in particular and the ER-Golgi system as a whole [8]. In broad terms, the nucleolus is a manufacturing, processing, and distribution center for certain macromolecules. The same overall description applies to the ER- Golgi system, where cargo proteins are synthesized (ER) and then processed covalently in a series of membrane-bound cisternal layers (Golgi stack) before being released to their final destinations.

Most of these cargo proteins are extensively glycosylated. The glycosyltransferases catalyzing the successive steps of this biosynthetic pathway are strategically located in successive cisternae of the stack: inner sugars in the structure are added at the entry face (termed "*cis*") of the stack; sugars in the middle being added in middle ("*medial*") cisternae, and outermost sugars being added at the other ("*trans*") end [7]. The cargoes depart the Golgi stack in separate membrane carriers, sorted according to destination (plasma membrane, lysosome, secretory storage vesicle etc.).

The ER and Golgi are tandem dynamic steady-state structures many of whose constituents are constantly inter-changing [9]. The luminal content of the ER constantly spills into the Golgi, and is recaptured by retrograde transport. Rather than staying in place, the

glycosyltransferases and most other resident proteins move rapidly within the Golgi and may even return during their lifetimes to the ER [9]. The cisternae themselves disassemble into much smaller vesicles during cell division, and these then reassemble in each daughter cell afterward [7] much like nucleoli. Analogous but even more complete disassembly of the Golgi is rapidly triggered by two classes of drugs acting via distinct pathways, and the Golgi reappears within minutes after the drug is removed [10,11].

This unusually plastic behavior has long been puzzling. How can a structure like the Golgi stack have steady-state polarity while most or even all of its components are so fluidly interchanging? How can it be so dramatically elastic, and like a stretched spring always return to its original state?

The simplest explanation would be that the Golgi, like the nucleolus, is fundamentally a liquid with a phase-separated internal organization. To illustrate (in an intentionally over-simplified manner) how this could work, imagine (Fig. 1) two distinct protein condensates (composed of proteins A and B, respectively), each of which phase separates into droplets that do not mix with each other. Imagine further that the A droplets and the B droplets adhere to and spread upon each other. This will be the case if the interface of A with B is lower in energy than the interface of either with water, which is expected if A and B have broadly similar chemistry and packing. Add a third (or more) analogous droplet (C) which can only spread on B, and the result will be a multilayered liquid which self assembles in a prescribed order. Each layer would be analogous to a Golgi cisterna, apart from the absence of a membrane. Layering in a prescribed order is analogous (in two dimensions) to the envelopment of one droplet by another that underlies the reconstitution of nucleolar organization [6]. There are many variations on this way of thinking. This includes additional "linker" protein components that could favor prescribed layering (by

stabilizing A-B but not B-C interfaces and so on) and the potential requirement for a lipid bilayer surface in some cases to orient the proteins for multi-phase separations (see third from last paragraph below).

What class of abundant Golgi constituents, if any, could comprise A, B, C etc. to enable such bulk phase separations? In the above example I chose proteins, but the Golgi also has abundant lipid bilayers and polysaccharides. Polysaccharides can be ruled out as a primary driver because they are sequestered from the cytoplasm in the luminal spaces of cisternae; the active principle would need to be exposed to the cytosol for regulation as in the cell cycle. Lipids are unlikely the prime mover because they are already phase-separated into liquid-crystalline bilayers by far stronger forces than would separate most proteins. Cargo proteins can be ruled out, among other reasons, because when the Golgi is emptied of cargo by blocking protein synthesis its structure remains intact.

The ideal candidate would be an abundant family of structurally related proteins on the cytoplasmic side of the cisternal membrane, different members (A, B, C etc.) being concentrated at different cisternae. Their structural similarities would favor adhesion, but their differences could favor de-mixing from each other under appropriate conditions to form separate liquid sub-compartments, as in the nucleolus. The liquid crystalline surfaces of A, B, C etc. would function as selective filters, like the hydrogel within nuclear pores [2], concentrating and incorporating proteins and vesicles they bind while excluding all others above a cutoff size. This would nicely explain the long-noted ~50 nm “zone of exclusion” of ribosomes from the Golgi membrane [12].

I have just described the “Golgins” - by far the most abundant group of compartmentally-specific peripheral membrane proteins of the Golgi [13]. The members of this family of related and evolutionarily-

conserved proteins assemble into long coiled-coils that are periodically interrupted to afford flexibility. The coils typically have 2- 4 helices and range in length from 50 – 400 nm when fully extended [14]. These rods are consistently oriented by attachment to cisternae where simple calculations suggest their local concentrations may reach the μM range at which many intrinsically disordered proteins are known to form liquid droplets in physiological buffers.

The Golgins are known to function as vesicle "tethers" [14]. The swarm of 50- 100 nm diameter transport vesicles that surrounds the Golgi are captured and retained by Golgins. Each type of vesicle is marked by a specific GTPase (Rab protein) and contains unique v-SNAREs. Vesicles are captured when its Rab protein binds to a specific, cognate tether. This enables the v-SNAREs to zip up with their cognate t-SNAREs, resulting in delivery of the cargo by membrane fusion [14].

While this understanding of Golgin function is undoubtedly correct, is it possible that it is a mere shadow of a fuller truth? For the sake of clarity, I will illustrate with a simple thought experiment. First, let us form a multi-lamellar liquid crystalline "proto-Golgi" in vitro by mixing pure Golgins A, B, and C , ordinarily present in the *cis*, *medial* and *trans* Golgi cisternae, respectively. Now, we will introduce this self-assembled "proto-Golgi" into the cytoplasm of a living cell. Each Golgin should now capture its cognate vesicles, obtained from the readily available supply in the cell. For example, Golgin A will bind vesicles containing the cognate *cis*-RabA, Golgin B will bind vesicles bearing the cognate *medial*-RabB, and so on, each thereby targeting to the cognate lamella of the Golgin liquid crystal. There, it would be expected to burrow through, chromatograph along, or otherwise traverse the local Golgin barrier, exactly as it would do in a natural Golgi. Once on the other side, these vesicles can fuse homo-typically with each other giving rise to compositionally distinct *cis*, *medial* and *trans* cisternae that interleave the appropriate liquid crystalline layers of Golgins. In broad

terms, this would be analogous to the passage of imported proteins across the nanoscale hydrogel that comprises the core of nuclear pores [2].

In this example the vesicles are provided by the cell, and these came from endogenous membranes. Each vesicle contained all the information (SNAREs, Rabs, etc.) needed to target correctly i.e. to propagate membrane compartmental specificity. On the other hand, the proto-Golgi contained all the complementary information needed to spatially organize these membrane compartments into a *cis-medial-trans* stacked scaffold. The former type of (membrane) information is thereby extra-genomically and generationally passed on from membrane to membrane following the now canonical dictum of Günter Blobel [15] "*omnis membrana e membrana*" in a system which cannot provide for *de novo* membrane self-assembly. By contrast, the latter and complementary type of (spatial) information would be directly encoded in genomic amino acid sequences that spontaneously self-assemble by multi-phase separation.

This is a new and complementary idea which in no way contradicts or diminishes the established one. It does however open interesting new possibilities for thinking about the evolution of organelles in which condensates template membranes, rather than the other way around. Our hypothetical proto-Golgi should thus "boot-up" and be fully functional, soon indistinguishable from its endogenous counterparts. In many ways, this would recapitulate what Warren and colleagues observed 25 years ago when they reconstituted post-mitotic Golgi re-assembly in cell-free extracts, an era during which they also discovered the first Golgins and suggested that they form a part of a solid multivalent "Golgi matrix" that is retained after detergent extraction and organizes the stack [7]. What is new is the idea that this "matrix" is self-organizing, liquid crystalline in nature, and may not even require

membranes to spontaneously recapitulate the fundamental outlines of Golgi architecture.

Before getting too carried away, one must admit that, based on their minimal content of low complexity sequences (in fact most Golgins do have what may be non-classical intrinsically disordered domains at their membrane-distal termini) or other known features such as high domain valance that favor phase-separation into droplets [16], there would seem to be very little to recommend Golgins. But still, they are worth considering because there may be many unappreciated ways in which phase separation could emerge in two dimensions from a multiplicity of low energy interactions (each just several times *kT*) to form liquid crystals that might not be as powerful in three dimensions [17]. In other words, the rules for surfaces may differ from those already established for droplets. For example, numerous weak lateral interactions patterned along the length of rods could favor lateral liquidity while maintaining vertical register when the rods are attached in the same orientation at high local concentrations on surfaces, as the Golgins typically are.

In summary, Golgins would still function in a limiting way as tethers to capture cognate vesicles, but they would now have a far more fundamental role, actually encoding the three dimensional architecture of the Golgi solely on the basis of their amino acid sequences - essentially as a continuation of protein folding. By extension, the many analogous rod-like tether proteins attached to membranes throughout the cell may also have deeper roles in generating and maintaining sub-cellular organization based on their intrinsic physical chemistries.

Function in biology dictates form. Why then should nucleolus and ER-Golgi, two systems with analogous manufacturing, processing, and distribution tasks, assume such different forms, the one being spherical and the other lamellar in nature? The nucleolus makes ribosomes,

which though very abundant are very long-lasting and are slowly and continuously manufactured. This enables RNA biosynthesis to take place within the core of the droplet, allowing a simpler spherical-based geometry to work for sequential processing. The secretory pathway is necessarily lamellar because of the massive surface area required for protein sequestration into the ER lumen. This spatial separation of manufacturing from processing requires a large surface area. Biosynthesis can no longer be accommodated at the center of a sphere, a basic constraint that likely dictates the overall lamellar arrangement of the ER-Golgi system, which may well be organized overall as one continuous system of adherent liquid protein and lipid condensates.

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Figure Legends:

Figure 1. Conceptual illustration of how a hypothetical “proto-Golgi” could spontaneously coalesce as a multi-phase system of liquid or liquid-crystalline of adherent proteins (A, B, C etc.) possibly facilitated by orientation on lipid bilayer surfaces (not shown). The architecture of a proto-Golgi could be specified entirely by the amino acid-sequences of its constituent proteins, and thus be directly encoded in the genome. It is suggested that the Golgins are the most likely candidates for A, B, C etc. in the Golgi stack. See text for details. This is an extreme case chosen for didactic reasons.

Figure 2. (A) Conceptual illustration of how a “proto-Golgi” could acquire specific *cis*- *medial*-*trans* processing compartments based on established mechanisms of vesicle tethering by rod-like Golgins and SNARE-dependent membrane fusion. Each Golgin tether binds its cognate vesicle via its cognate Rab[GTP], partitioning into and crossing the “zone of exclusion” barrier created by the Golgin liquid phase. Having crossed, the vesicles can now fuse homo-typically utilizing their endogenous v-SNAREs and t-SNAREs activated by NSF ATPase. This makes facilitated diffusion across the barrier irreversible, and results in a specific cisternal sub-compartment in between the layers of Golgins Note that there are typically multiple cognate Rab[GTP] binding sites along the length of the tether [14]. This process bears overall similarity to the import of Nuclear Localization Signal (NLS)-containing cargo into

the nucleus via nuclear pores (B). NLS-tagged cargo are captured by Nuclear Transport Receptors (NTRs) involving a cousin of Rab[GTP], Ran[GTP]. The cargo-bearing complexes partition by cognate binding to FG repeat motifs, themselves present at a high density along the length of this class of nucleoporins, which are phase separated into a hydrogel on the basis of their intrinsically disordered FG repeats. The Cargo-NTR-Ran[GTP] complex can cross the "zone of exclusion" because it specifically locally dissolves the hydrogel barrier (it may well be that the vesicles in 'A' also locally perturb the cognate Golgin phase). After crossing, GTP is cleaved and the Cargo dissociates, rendering what would otherwise be a bi-directional process of facilitated diffusion irreversible [2]. Wavy lines represent Golgins in 'A' and FG-repeat-containing Nups in 'B'. Small solid circles placed on the wavy lines represent cognate binding sites for Rab[GTP] in 'A' and for NTR in 'B'.