Fortunately, new tools in Drosophila are making its solution accessible.

References

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Organelle Dynamics: A Tale of Fusing Nucleoli

Recent experiments on nucleoli suggest that their dynamic behavior is liquid-like with common fusion events and that the surrounding actin plays an active role in these dynamics.

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Understanding the basic principles of self-assembly in biological systems, and the connection between underlying molecular activities and the resulting macroscopic organization are two of the greatest challenges in biology and biophysics [1]. One particular example — fundamental for cell growth and development — is provided by the RNA–protein assemblies found in the nucleoplasm of cells. Despite experiments on nucleolar fusion of somatic cells dating back almost a hundred years, the assembly dynamics of such complexes are still poorly understood [2].

The nucleolus is an intriguing non-membrane-bound assembly, made up of proteins and nucleic acids, that resides in the nucleus. It is important for the transcription and assembly of ribosomal RNA. Understanding the assembly behavior of nucleoli, and what regulates their size and shape, is key to understanding the underlying causes of diseases such as Huntington’s disease [3] and spinal muscular dystrophy [4].

Recent electron microscopy and fluorescence imaging studies show that nucleoli contain complex structures [5]. It is natural to expect that the aggregation of proteins and RNA will lead to irregular complex assemblies, similar to those seen in amyloid plaques found in Alzheimer patients [6]. However, nucleoli are often observed to be spherical, which raises an important question. How do nucleoli self-assemble into these isotropic structures?

Now, in a recently published study, Brangwynne et al. [7] have used the germlinal vesicles of amphibian oocytes to probe the fundamental nature of nucleoli assembly, and understand the principles that regulate nucleolar structure and dynamics. Using fluorescence imaging of GFP-tagged nucleolar proteins, and differential interference contrast microscopy, these authors tracked and analyzed large ensembles of nucleoli. Their observations show that nucleoli are mostly isotropic in shape, much like liquid droplets, with a power law size distribution.

These observations raised even more questions. If the nucleoli are indeed liquid-like, one should expect to see fusion events between two or more nucleoli, leading to the observed different sizes. Would the volume of the nucleoli be conserved just like in living cells? What are the volume distributions? Would the nucleoli undergo fission events? What is their effective surface tension?

To test the hypothesis that nucleoli are formed as the result of a liquid-like aggregation process [8], Brangwynne et al. [7] first performed Monte Carlo simulations of diffusive aggregation of droplets, and obtained a power law distribution of sizes with the same exponent as they measured for nucleoli. An important property of power-law distributions is scale invariance, i.e. the distribution is independent of the particular scale chosen. An ensemble
of spheres with this type of size distribution is illustrated in Figure 1A. Such power law distributions are commonly observed in many natural and man-made systems; examples range from the distribution of household income to that of animal metabolic rates. As Brangwynne et al. [7] argue in their paper, this suggests the lack of a characteristic nucleoli size in oocytes.

Brangwynne et al. [7] continued their study with a series of experiments in which they not only observed individual fusion events, but also induced fusion events using microneedles. Their results showed that two or more nucleoli often fuse (as illustrated in Figure 1B), and that the surface tension is sufficiently large to result in spherical nucleoli with conserved volume. While they have never observed nucleoli splitting into two or more pieces in their experiments, occasionally they observed a ‘pinch-off’ behavior followed by rupture of the liquid bridge, characteristic of viscous fluids [9].

Using the analogy to Newtonian liquid droplets suspended in a lower viscosity medium, and measuring the characteristic time it takes two nucleoli to fuse, Brangwynne et al. [7] estimated the effective nucleoli surface tension. They found it to be on the μN/m range, resulting in an effective viscosity six orders of magnitude larger than the viscosity of water. In an earlier study, Brangwynne et al. [10] showed that germ granules in Caenorhabditis elegans also exhibit liquid-like properties, with, however, an order of magnitude larger apparent viscosity. It remains to be seen whether this liquid-like behavior is a common feature of all RNA–protein assemblies found in the nucleoplasm and cytoplasm.

While the majority of the nucleoli that have been observed by Brangwynne et al. [7] exhibit liquid-like viscous relaxation behavior, a sub-population of nucleoli (<5%) had irregular shapes and did not relax to spheres. One possible hypothesis, put forward by Brangwynne et al. [7], is that nucleolar fusion is driven by an active metabolism, and the disruption of this mechanism, in possibly damaged germinal vesicles, leads to loss of liquid-like behavior. To test this hypothesis, they depleted ATP in the germinal vesicles. The resulting nucleoli exhibited slower relaxation dynamics following fusion, with an apparent viscosity that is an order of magnitude larger than in control cells. Brangwynne et al. [7] suggest that actin, which is present in abundance, can act as a scaffold, giving both structure and stability to the nucleoli, and possibly set the rate of nucleoli diffusion and fusion.

Measurement of the diffusion of nucleoli in germinal vesicles, both in control and in actin-disrupted cells, could possibly help clarify the role of actin further and verify that the system is indeed in a diffusion-limited regime as suggested by the authors. An extensive characterization of this behavior could provide valuable information about the structure of the scaffold provided by actin.

This simple liquid-like picture, while very attractive, was still an apparent contradiction with the existing electron microscopy studies that reported complex core-shell structures. In order to reconcile these two pictures, Brangwynne et al. [7] also visualized fluorescently labeled nucleolar sub-structures during fusion events. They found that these sub-structures do not form well-defined assemblies, but instead act like nucleation sites that can facilitate the clustering of nucleolar material to form droplets. The number of these nucleolar sub-structures is also reported to be conserved during fusion events [7].

An open question is the motility of these nucleolar sub-structures. Do they move as one would expect from beads in a dense fluid? If they do, is the motion diffusive? Sub-diffusive? Recently, the tracking of existing liquid domains within lipid bilayers has been used to measure the effective membrane viscosity of these bilayers [11]. If these nucleolar sub-structures are indeed motile, characterization of their dynamical behavior could similarly provide valuable information about the liquid-like structure of the nucleoli.

It is intriguing to consider nucleoli as dynamic liquid-like structures, and the experimental observations of Brangwynne et al. [7] strongly support this hypothesis. However, several questions remain unanswered — for example, if nucleoli are indeed liquid-like, why do they not fuse into one big structure? What is the role of the F-actin network surrounding the nucleoli? Further experiments are necessary to answer these questions. The significance of a power-law nucleoli size distribution and the functional role of nucleolar fusion are other open questions that remain to be explored.

References
Notch Signaling: A Role in Sleep and Stress

The molecular pathways regulating sleep remain poorly understood. Studies in this issue demonstrate a role for Notch signaling in sleep regulation as well as stress response in both Caenorhabditis elegans and Drosophila.

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It has become clear in recent years that signaling pathways used for development also play roles in regulation of nervous system physiology. Notch signaling, which has classically been studied in cell fate specification during development [1], is increasingly recognized to play important roles in the adult nervous system [2–5]. Ligand activation of NOTCH causes the cleavage of the NOTCH intracellular domain, which then translocates to the nucleus to regulate gene transcription, thus impacting development or behavior [6]. Two articles published in this issue of Current Biology [7,8], reveal a role for this key signaling pathway in the regulation of sleep and sleep-like states in both Caenorhabditis elegans and Drosophila.

The discovery of a role for Notch signaling in the regulation of C. elegans quiescence and sleep-like behavior began with an unexpected observation. The authors had been studying the function of OSM-11, which is a founding member of a family of Notch co-ligands with DOS (Delta OSm) domains [9]. They noticed that overexpression of OSM-11 causes adult animals to stop moving and feeding. This quiescence induced by OSM-11 is dependent on lin-12 and glp-1, the two C. elegans genes encoding Notch receptors.

Behavioral quiescence under standard laboratory conditions normally occurs only during lethargus, a stage with sleep-like behavioral features that is associated with each of the four larval molts [10]. Therefore, observing the induction of adult quiescence by OSM-11 overexpression and Notch signaling led Singh and colleagues [7] to test for a role of this signaling pathway in lethargus behavior. The behavior of OSM-11 overexpressing animals is similar to that of worms in lethargus in that they both have elevated arousal thresholds, a property seen in sleep. Further, OSM-11–induced quiescence is dependent on the genes egl-4, deg-3, and ceh-17, all of which regulate lethargus. The egl-4 gene encodes a cGMP-dependent protein kinase required for normal molting quiescence and sleep-like behavior [10], whereas deg-3 and ceh-17 are required for the function of ALA, a single interneuron that is required for normal expression of lethargus quiescence [11]. Loss of OSM-11 in combination with loss of another DOS domain protein called OSM-7 results in severely reduced molting quiescence and increased responsiveness during the normally sleep-like lethargus period [7]. These experiments thus demonstrate that Notch signaling and, in particular, DOS domain Notch ligand signaling, promotes sleep-like behavior during lethargus.

However, this simple model becomes more complicated when considering the phenotypes of osm-11 and osm-7 single mutants and of single mutants in each of the two worm NOTCH receptors. Surprisingly, these mutants show increased (not decreased) total lethargus quiescence. But the authors also show that every genetic manipulation that reduces Notch signaling results in a lowering of the normally elevated arousal threshold during lethargus. Their careful analysis underscores the importance of considering not only the quantity but also the quality of sleep in invertebrate model organisms.

The second paper published in this issue of Current Biology examines the role of Notch signaling in sleep regulation in Drosophila melanogaster [8]. The evidence that Drosophila sleep is fundamentally similar to mammalian sleep is extensive and includes genetic, molecular, and pharmacological observations [12]. Seugnet and colleagues [8] focus their analysis on the phenomenon of increased sleep (‘sleep rebound’) following sleep deprivation. Sleep rebound is thought to reflect increased sleep pressure driven by a sleep homeostatic process.

Seugnet and colleagues begin with the observation that the transcription factor bunched, which has been shown to negatively regulate Notch signaling [13], is upregulated following sleep deprivation. They build upon this initial observation, exploring the role and cellular basis of Notch signaling in homeostatic regulation of sleep. They show that overexpression of the NOTCH ligand DELTA in mushroom bodies, a structure implicated in learning/memory as well as sleep regulation [14,15], reduces sleep rebound. Similarly, a Notch gain-of-function allele also exhibits reduced sleep rebound. Taken together, these data suggest that sleep deprivation normally suppresses Notch signaling (by upregulating bunched), and that this reduction of Notch signaling allows for the expression of sleep rebound.

The function of sleep is unknown but one popular theory posits that sleep modulates synaptic plasticity and learning and memory [16]. In previous work, the authors showed that sleep-deprived flies are defective in a specific associative learning task [17,18]. The authors show that increasing Notch signaling (by either overexpressing DELTA in mushroom...