55. Gene expression organization and Biomolecular Condensates

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Class overview

To properly regulate gene expression, cells must carefully coordinate many RNA processing events in both space and time. One way cells achieve this is through membraneless structures called **biomolecular condensates**, which help organize and concentrate RNA processing activities. Recent research suggests that these condensates form through **biological phase transitions**, creating uniquely dynamic microenvironments. These findings have sparked a growing field of study with important implications for both basic biology and disease, as many disorders involve disrupted condensates. In this class, we will explore the **biophysics of condensate formation**, their **proposed functions** in the cell, and the **experimental methods** used to study their physical and functional properties. We will also touch on the role of biomolecular condensates in disease and avenues for condensate-specific therapeutics.

Topics covered in this class will include:

1. The physicochemical basis for condensate formation: phase transitions and polymer chemistry
2. Biological functions for biomolecular condensates in gene regulation and beyond
3. Methods for studying biomolecular condensates
4. A case study: the nucleolus as a multiphase biomolecular condensate
5. Condensates in disease and "drugging" condensates
6. Critical analysis of evidence supporting different condensate assembly mechanisms

Class reading

Required reading:

Biomolecular Condensates: Organizers of Cellular Biochemistry – Banani et al. *Nature Reviews Molecular Cell Biology* (2017)
https://www.nature.com/articles/nrm.2017.7
Foundational overview of biomolecular condensates, their assembly principles, and their proposed functions

Suggested reading:

Phase Separation in Biology and Disease; Current Perspectives and Open Questions – Boeynaems et al. *JMB* (2023)
Overview from leaders in the field about current questions and perspectives in condensate research.

Fundamental Aspects of Phase-Separated Biomolecular Condensates – Ghosh et al. *Chemical Reviews* (2024)
https://pubs.acs.org/doi/full/10.1021/acs.chemrev.4c00138
In-depth overview of the physics of phase separation

Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates – Alberti et al. *Cell* (2019)

https://www.sciencedirect.com/science/article/pii/S0092867418316490
Proposes guidelines for rigorous experimental characterization of phase separation processes in vitro and in cells, discusses caveats of common experimental approaches, and points out experimental and theoretical gaps in the field.

A conceptual framework for understanding phase separation and addressing open questions and challenges – Mittag, T. & Pappu, R.V. *Molecular Cell* (2022)
https://www.sciencedirect.com/science/article/pii/S1097276522004853?via%3Dihub
Addresses controversies in phase separation.

Biomolecular Condensates and Cancer – Boija et al. *Cancer Cell* (2021)
https://www.sciencedirect.com/science/article/pii/S1535610820306103?via%3Dihub
Overview of condensates and their relation to malignancy.

Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing - Patel, A., et al. *Nature Reviews Molecular Cell Biology* (2021)
https://www.nature.com/articles/s41580-020-00326-6#Sec26
An overview of how condensates and their assembly features are involved in cellular stress responses and diseases of aging.

Modulating biomolecular condensates: a novel approach to drug discovery – Mitrea et al. *Nature Reviews Drug Discovery* (2022)
https://www.nature.com/articles/s41573-022-00505-4
A framework for how condensates may be approached as "druggable" targets.

Class activity

It has been widely proposed that the nucleolus, one of the largest and best-studied biomolecular condensates in the cell, is a multilayered condensate composed of three layers that form via liquid-liquid phase separation (LLPS). It has been strongly suggested (and often assumed) that the dynamic, liquid-like assembly of the nucleolus is important for its core molecular function in ribosome assembly. This idea contrasts with what we know about ribosome biogenesis from years of molecular work demonstrating that it is a highly ordered and structured process.

Therefore, I pose a core question to the class: **does current experimental evidence support that the nucleolus forms via LLPS and that this is important for its function?** What alternatives may there be to this hypothesis?

To critically explore this controversy, the class will be divided into two groups, one that will argue for and one that will argue against the following:

1. LLPS as the assembly mechanism of the nucleolus
2. The importance of LLPS in ribosome biogenesis

We will conclude with future perspectives for unraveling nucleolar form and function. **All students** should come prepared with a proposed next experiment for better understanding the assembly features of the nucleolus and/or how they relate to ribosome assembly.

Below are some resources for both perspectives, but these are simply a starting point. It is encouraged that students critically explore the literature independently to craft their argument.

Papers SUPPORTING Nucleolus Formation by LLPS:

Coexisting liquid phases underlie nucleolar sub-compartments - Feric, M., et al. (2016). *Cell*.
Mitrea, D.M., et al. (2018). "Self-interaction of NPM1 modulates multiple mechanisms of liquid–liquid phase separation." *Nature Communications*.
Yao, R.W., et al. (2019). "The nucleolus as a multiphase liquid condensate." *Nature Reviews Molecular Cell Biology*.

Papers AGAINST or QUESTIONING Nucleolus Formation by LLPS:

McSwiggen, D.T., et al. (2019). "Evaluating phase separation in live cells: diagnosis, caveats, and functional consequences." *Genes & Development*.
Tartakoff, A., et al. (2022). "The dual nature of the nucleolus." *Genes & Development*.
Putnam, A., et al. (2023). "RNA granules: functional compartments or incidental condensates?" *Genes & Development*.

Group A – FOR/Supporting Nucleolus Formation by LLPS:

Afroz, Jalwa – LEADER
Mutaher, Mohammed
Volpe, Christina
Sussman, Carleigh
Magnus, Karina

Group B – AGAINST or QUESTIONING Nucleolus Formation by LLPS:

Beattie, Kai – LEADER
Li, Faye
Ahmed, Nibras
Pope, Eleanor
Styers, Hannah
McIlhenny, Lauren