An intracellular phase transition drives nucleolar assembly and size control

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Membranes spatially organize eukaryotic cells
Cells also contain membraneless compartments

Germ granules

Stress granules

*C. elegans* embryo

U2OS cells

Brangwynne et al 2009, Molliex et al 2015
Cell size changes through growth and development

Reductive division

Hypertrophic growth

L1 larva → Adult

S. Uppaluri
How do cells establish and dynamically regulate spatial organization?

How do membraneless organelles assemble?

How do cells coordinate and control the size of their organelles?
The nucleolus is a membraneless organelle

- Assembles around ribosomal DNA
- Synthesizes ribosomal subunits

*C. elegans* embryo

Nucleoli (FIB-1::GFP)
Cell membrane (mCherry::PH<sup>PLCδ1</sup>)

Cytoplasm

Ribosome

Nucleus

Nucleolus

rDNA
Membraneless organelles behave like liquids

Droplet fusion

Wetting and dripping

Brangwynne et al 2011, Brangwynne et al 2009
Purified components condense into droplets and gels in vitro

Multivalent domains

Low complexity sequences

[G/S]Y[G/S]

Hypothesis: membraneless organelles assemble via intracellular phase transitions

“Gas”

“Liquid”

“Solid”

Patel et al 2015

Weber and Brangwynne 2012
Phase diagram predicts behavior of a liquid mixture

Phase boundary

One phase, mixed

Two phases, separated

Temperature

Saturation concentration

Concentration

All Red

All Blue
Hallmarks of phase separation

1. Saturation concentration sets a threshold for droplet condensation

2. Size of condensed phase droplets increases with concentration
Concentration can be tuned by manipulating embryo size

Weber and Brangwynne 2015
Nucleolar size increases with concentration

Control

C27D9.1(RNAi)  ani-2(RNAi)  ima-3(RNAi)

Weber and Brangwynne 2015
Quantifying nucleolar size

Maximum nucleolar intensity per cell

\[ I_0 = I_1 + I_2 \]

Weber and Brangwynne 2015
Nucleolar size increases with concentration

\[ I_o = \alpha (C_n - C_{sat}) V_n \]

Phase transition model

- **C27D9.1(RNAi)**
- **Control**
- **ani-2(RNAi)**
- **ima-3(RNAi)**

Where:
- \( I_o \) is the max nucleolar intensity (a.u.)
- \( \alpha \) is a proportionality constant
- \( C_n \) is the nuclear concentration (µM)
- \( C_{sat} \) is the saturation concentration
- \( V_n \) is the nuclear volume (200 µm³)

Weber and Brangwynne 2015
Nucleolar size increases with concentration

Phase transition model

\[ I_o = \alpha(C_n - C_{sat})V_n \]

\( V_n = 200 \mu m^3 \)

- **C27D9.1(RNAi)**
- Control
- **ani-2(RNAi)**
- **ima-3(RNAi)**
Nucleolar assembly depends on cell lineage, embryonic stage

8-cell stage

4-cell stage

ABp

Nucleoli do not assemble in AB lineage until 8-cell stage

ABa

EMS

| Small, transient nucleoli in EMS |

Weber and Brangwynne 2015
Saturation concentration at the 4-cell stage

Max nucleolar intensity, $I_o$ (a.u.)

- **C27D9.1(RNAi)**
- **Control**
- **ani-2(RNAi)**
- **ima-3(RNAi)**

Nuclear Concentration, $C_n$ (µM)

- $C_{sat}^{4-cell}$
- $C_{sat}^{8-cell}$

Weber and Brangwynne 2015
Phase diagram for nucleolar assembly *in vivo*

Nuclear Concentration, $C_n$ (µM)

Temperature

Concentration

$\chi = \text{developmentally-regulated interaction parameter}$
Phase diagram for nucleolar assembly \textit{in vivo}

\begin{itemize}
\item \textit{C27D9.1} (RNAi)
\item Control
\item \textit{ani-2} (RNAi)
\item \textit{ima-3} (RNAi)
\end{itemize}

$\text{Max nucleolar intensity, } I_0 \text{ (a.u.)}$

$\text{Nuclear Concentration, } C_n \text{ (µM)}$

$V_n = 200 \text{ µm}^3$

Control

\textit{ani-2} (RNAi)

\textit{ima-3} (RNAi)
Nuclear concentration is ~constant during early embryogenesis

8-cell  16-cell  32-cell  64-cell

![Cell stages](image)

![Graph](image)

Integrated intensity (a.u.)

Embryos
Maternal load

Cell Stage
Onset of zygotic expression
Phase separation gives rise to organelle size scaling

\[ I_o = \alpha [C_n - C_{sat}] V_n \]

Max nucleolar intensity, \( I_o \) (a.u.)

Control
\( C_n = 0.13 \mu M \)

Nuclear Volume, \( V_n \) (\( \mu m^3 \))

Cell stage: 64 32 16 8

Weber and Brangwynne 2015
Summary of results

1. Nucleolar size increases with concentration (8-cell stage)

2. Saturation threshold for nucleolar assembly (4-cell stage)

3. Nucleolar size scales with nuclear size (8→64-cell stages)
Membraneless organelles
• increase the local concentration of enzymes and substrates
  - accelerate biochemical reactions
• can also sequester reactants to inhibit activity
Nucleolar size, activity correlate with cell growth and proliferation

Defects in ribosome biogenesis limit cell and organism size

SFP1 overexpression

Δsfp1

Misregulation of nucleolar size drives tumor growth

Benign

Malignant

Measuring nucleolar activity

Ribosome biogenesis
- Transcription
- Processing
- Subunit assembly
- Nuclear export

Fluorescence *in situ* hybridization

rDNA locus:
Large nucleoli produce more nascent transcripts
rRNA transcription increases with component concentration, nucleolar size.

AB lineage, 8-cell stage

RNAi condition: FISH integrated intensity (a.u.)
- C27D9.1
- WT
- ani-2
- ima-3

Nuclear concentration
*ncl-1* mutants have enlarged nucleoli

Yochem and Herman 2003

Increased levels of rRNA

Increased growth rate, body size

Total RNA
Nucleolar size predicts organismal growth rate

Uppaluri, Weber and Brangwynne 2016
Conclusions

• Supersaturation drives nucleolar assembly *in vivo*

• Phase separation gives rise to organelle size scaling

• Nucleolar size correlates with rRNA transcription and organismal growth
Thank you!

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