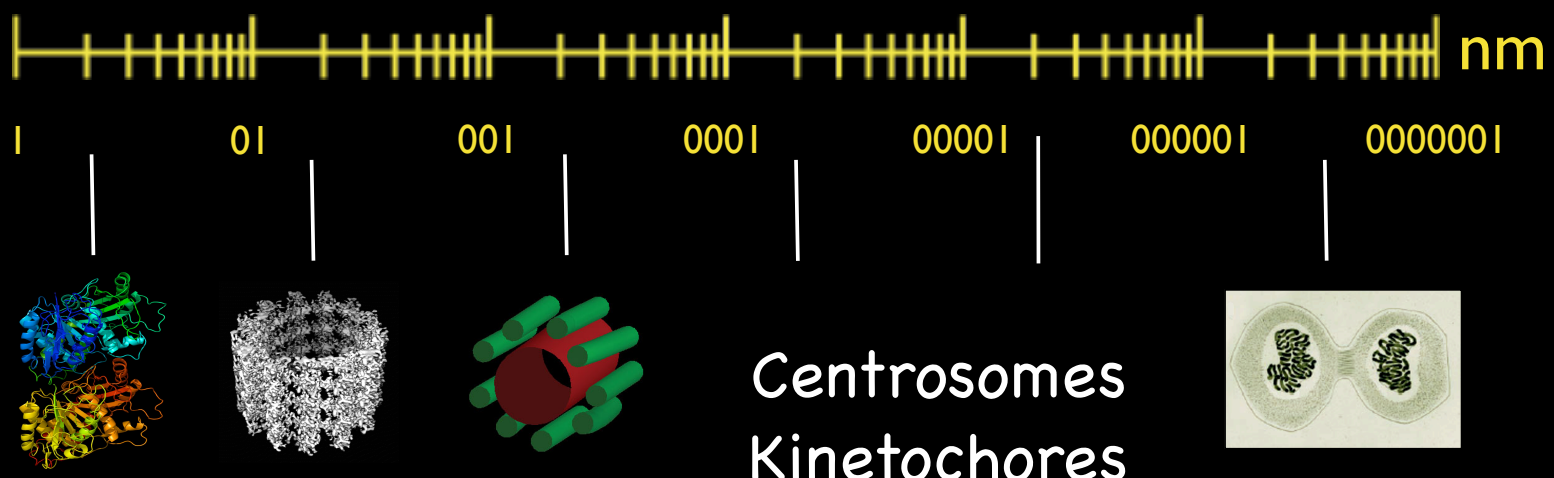
Formation of P granules

Tony Hyman

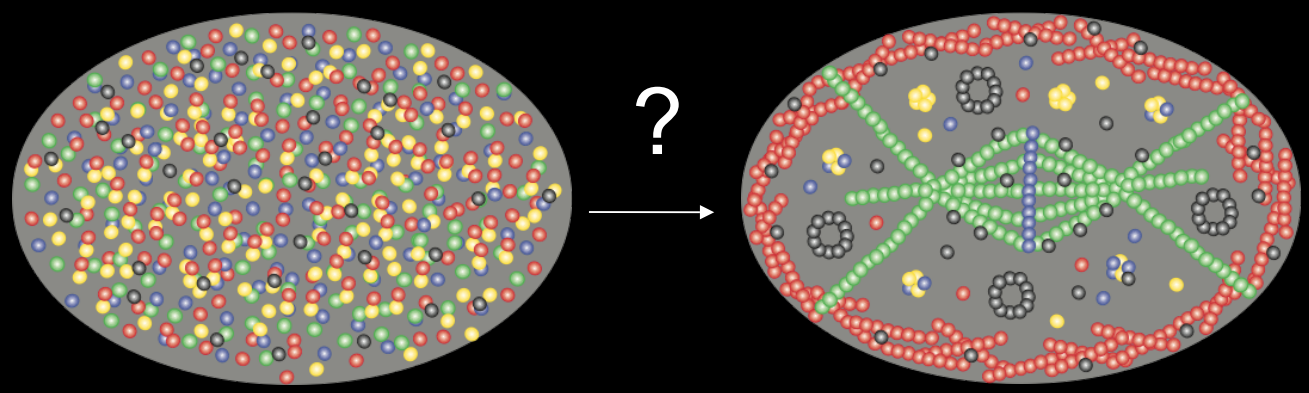
Max Planck Institute of Cell Biology
and Genetics
Dresden Germany

Large non-membrane bound compartments (organelles)

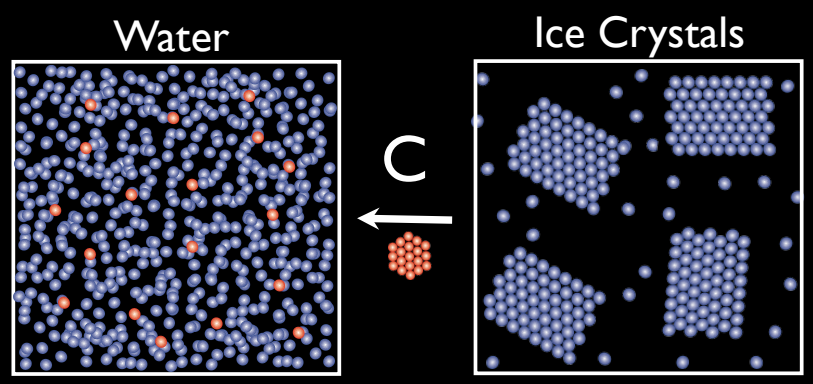


Centrosomes
Kinetochores
Protein/RNPs
Nuclear bodies

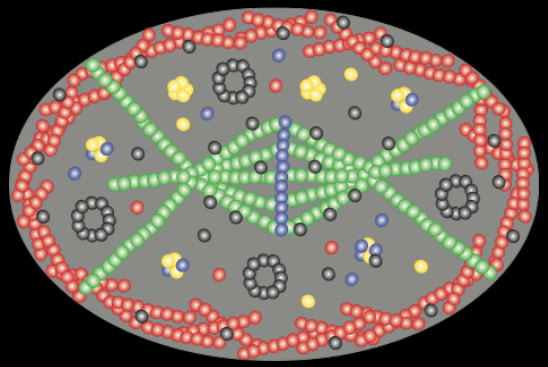
How to structure large non membranous bound organelles?

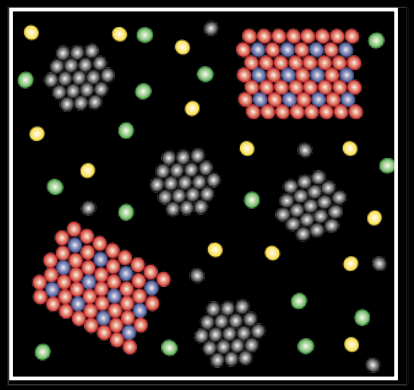


What can we learn from non-biological systems ?



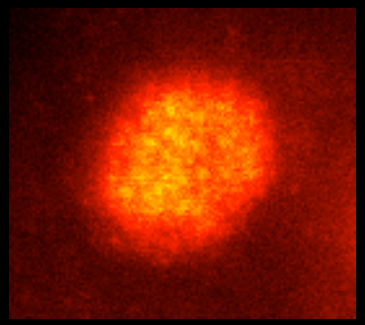
Do non-biological structures have anything to do with biological assembly?





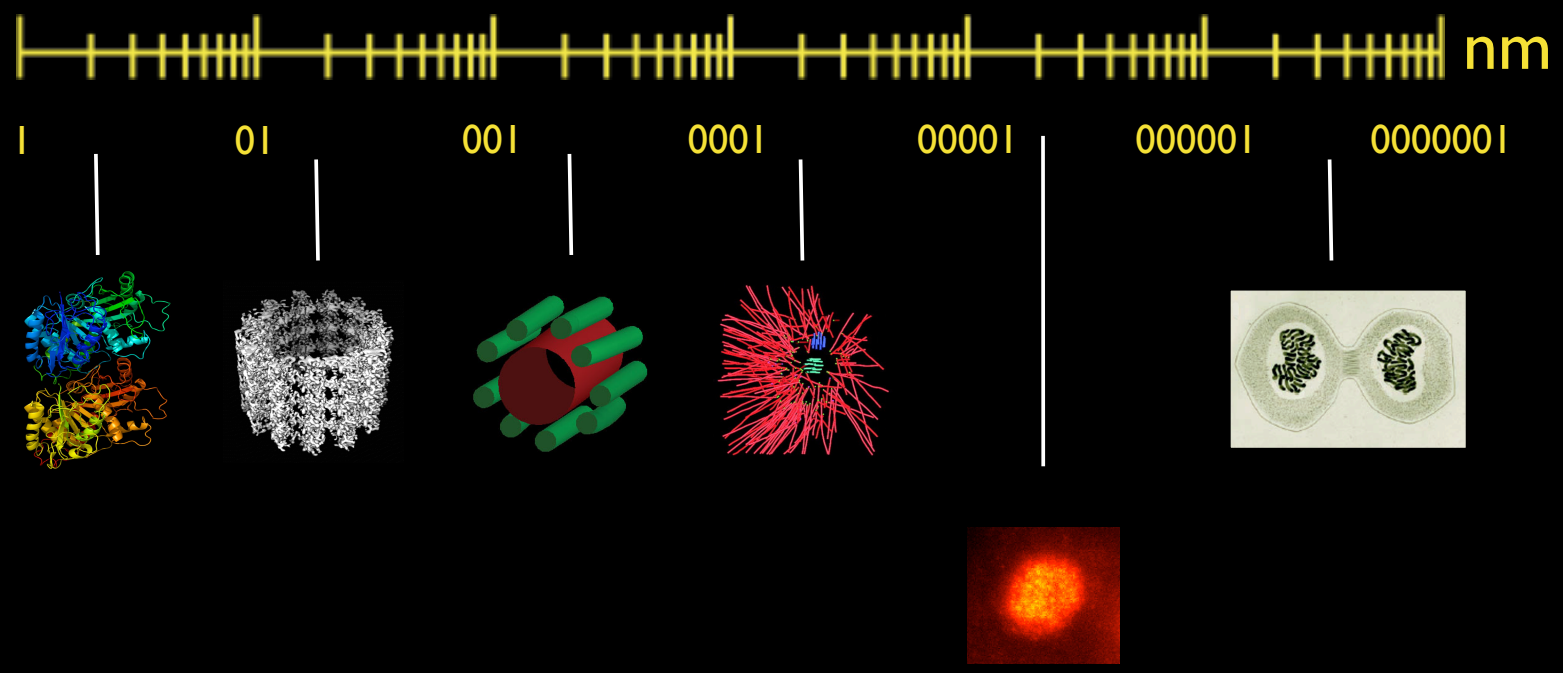
- **Cliff Brangwynne**
- Frank Julicher (Max Planck for physics of complex systems)
- Christian Eckmann,
- Carsten Hoege, Agata Rybyrska

P granules are cytoplasmic hubs of Proteins and RNAs

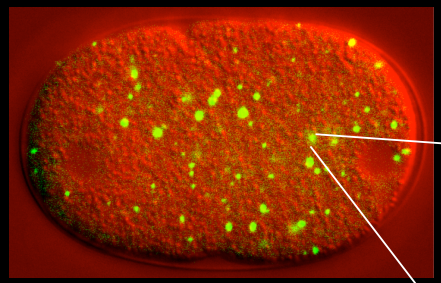


Generation..... of germ-line granules in early C. elegans embryos.
Strome S, Wood WB. Cell. 1983 Nov;35(1):15-25.

P granules can be large



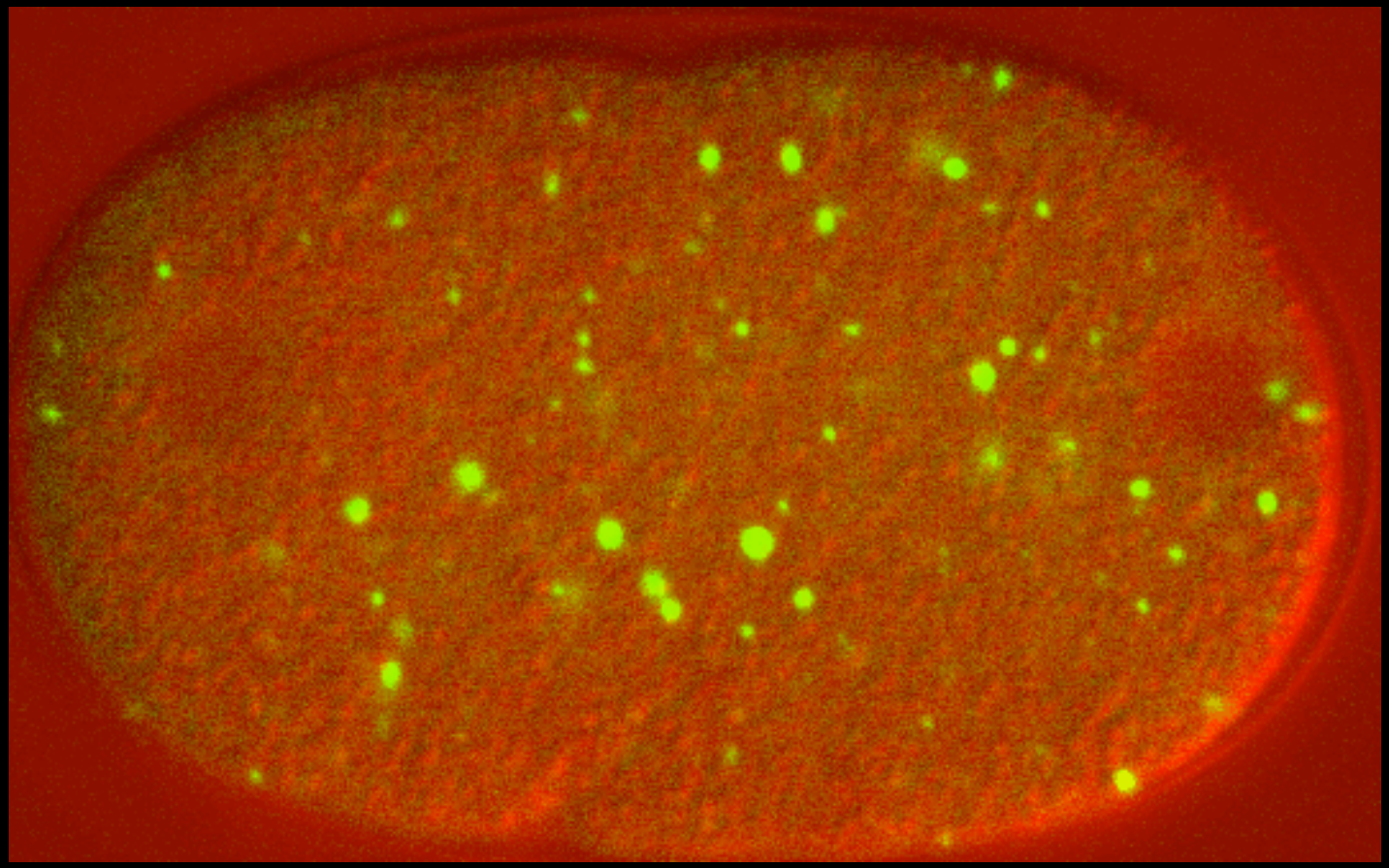
P granules are complex



P Granules
contain RNA &
RNA-binding proteins

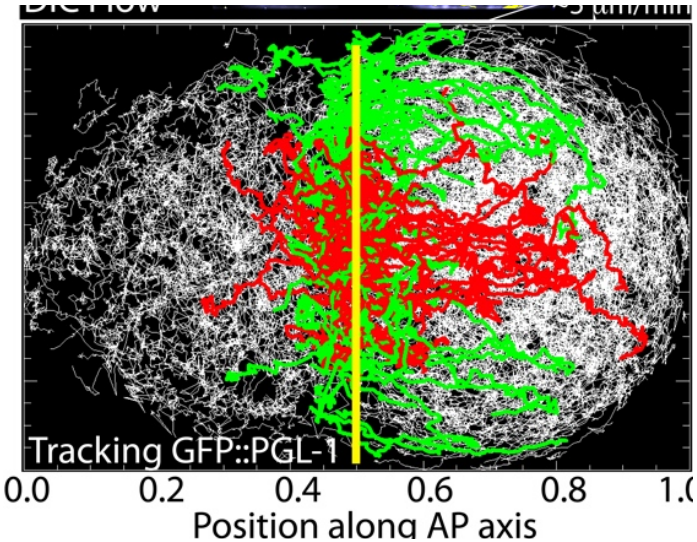
Believed to be important for totipotent state of germline.

P granules segregate to the posterior



Anterior **posterior**

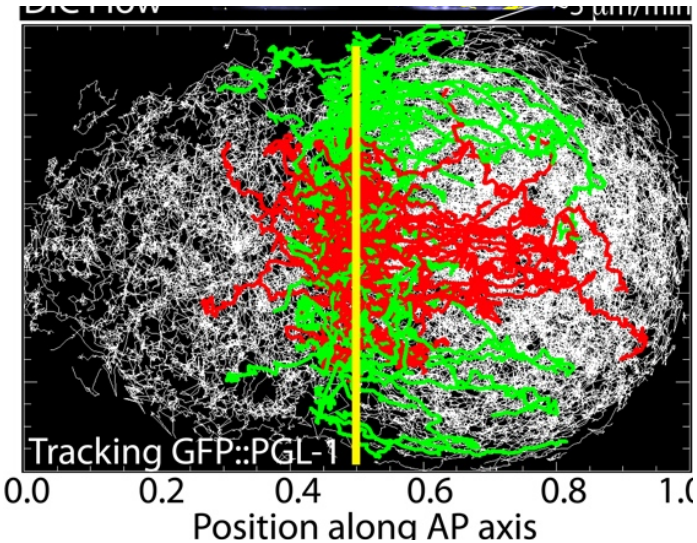
Can P granules can move by cytoplasmic flow?



3D particle tracking:

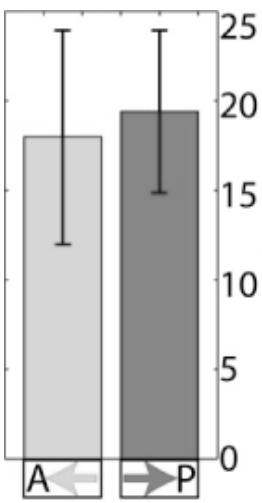
Cliff Brangwynne

P granules do not segregate by cytoplasmic flow



3D particle tracking:

*There is **no net flux** into posterior !!!*



Average number crossing middle of the embryo

What is the fate of individual P granules?

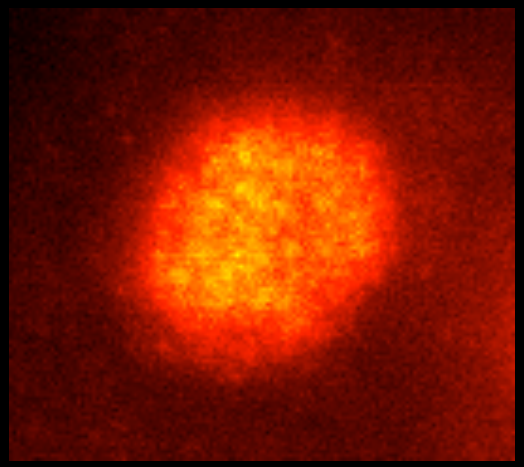
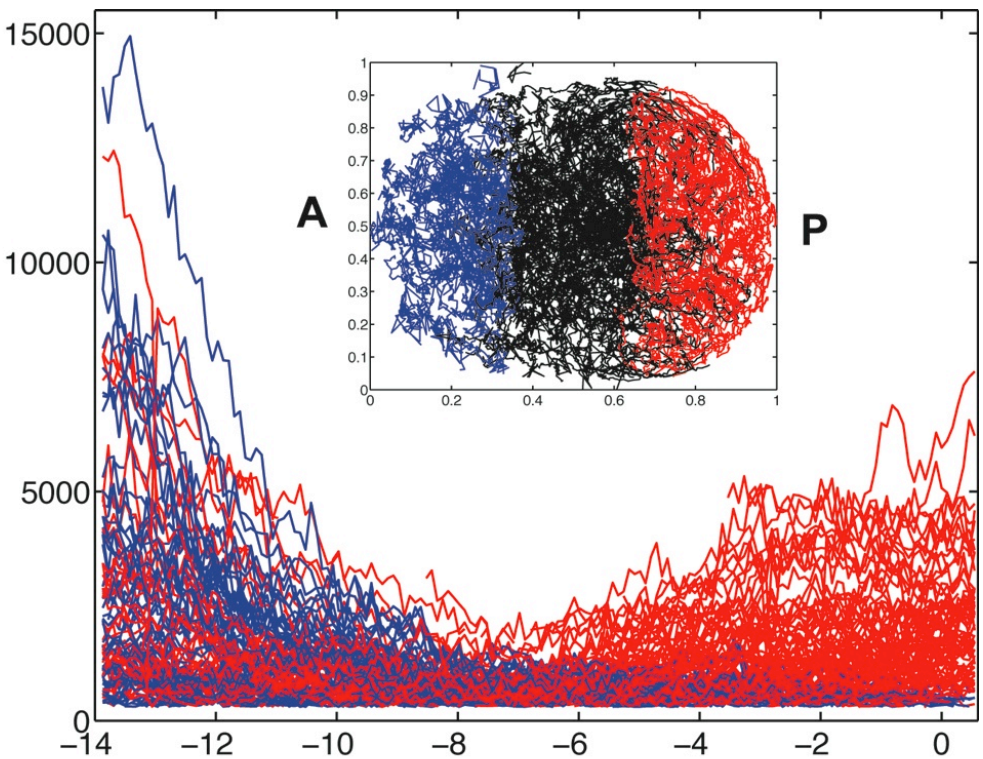


Image of P-granule by STED microscopy
with Stephan Hell

P-granules are dynamic

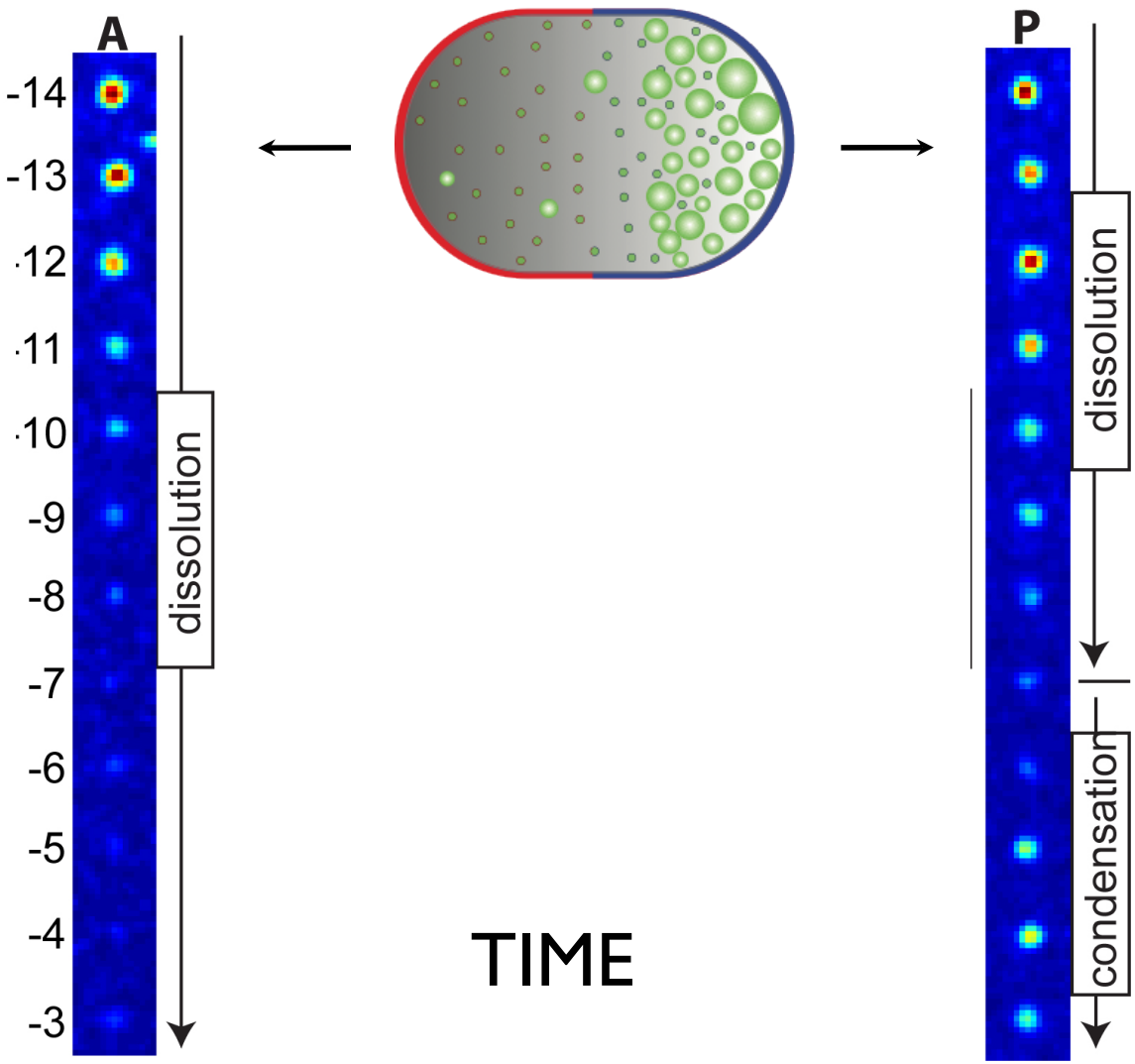
INTENSITY



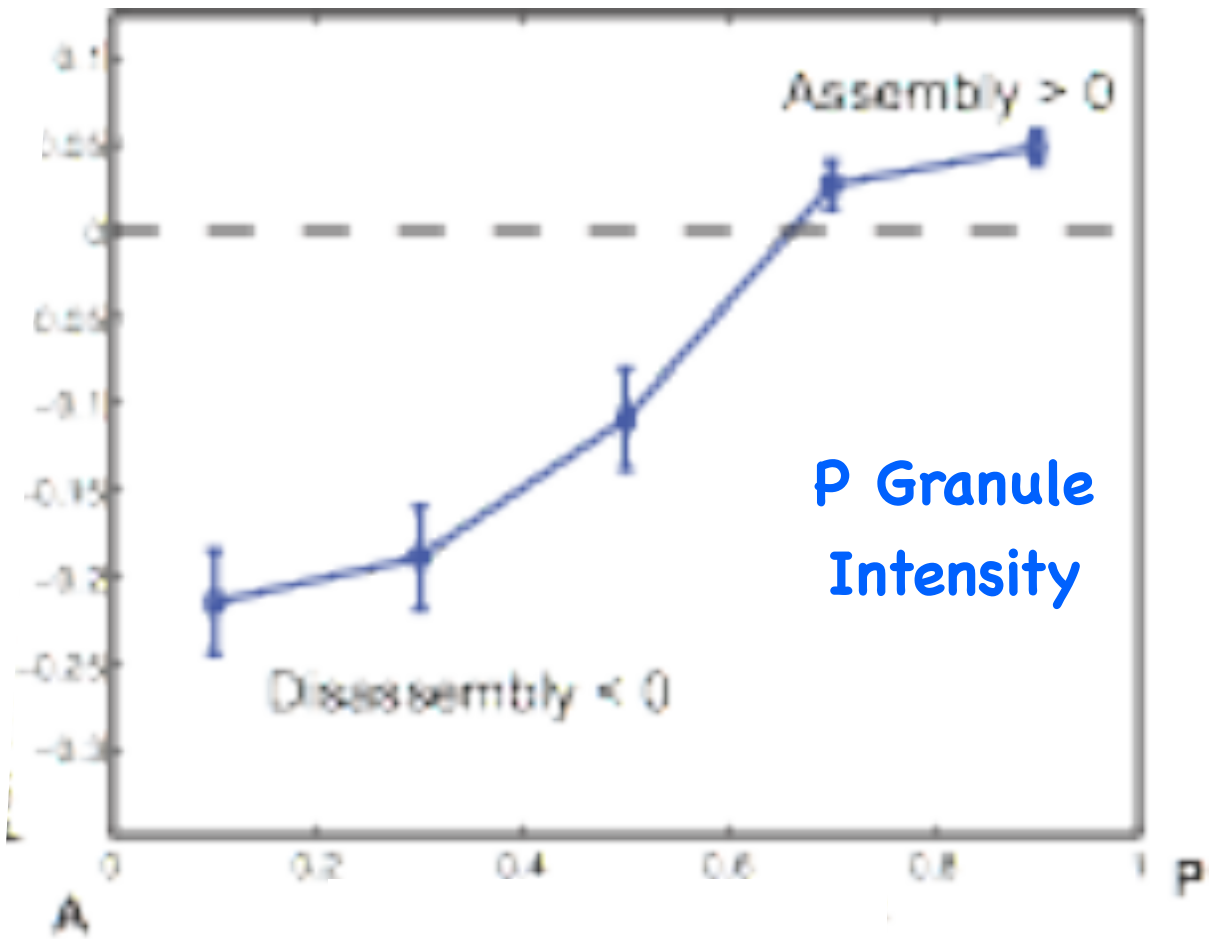
TIME

Brangwynne, Eckmann Julicher, and Hyman. Science, 2009

P granules dissolve at the anterior and condense at the posterior

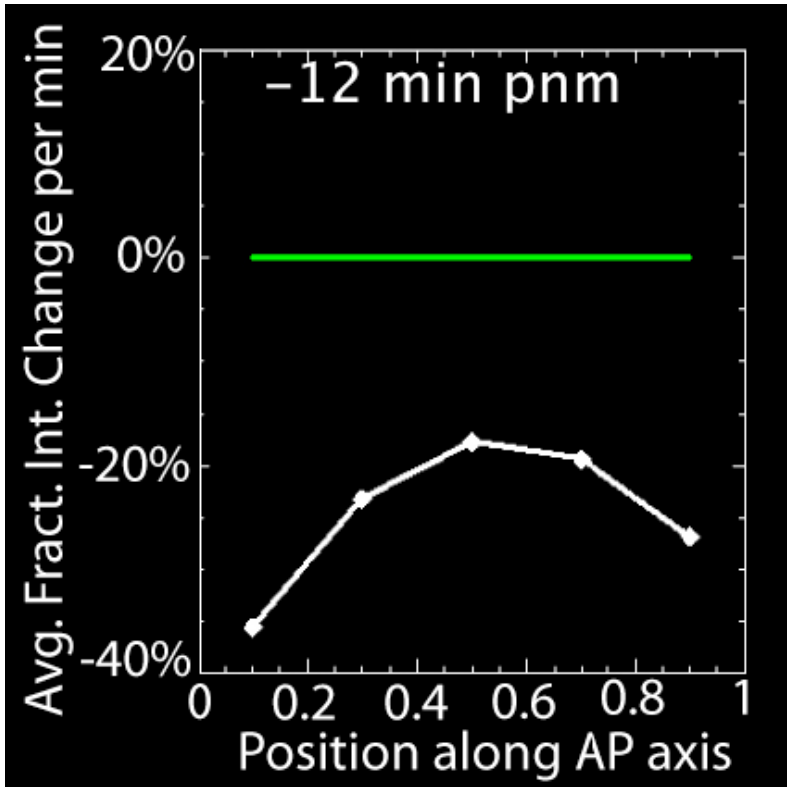


There is a gradient of P granule assembly/disassembly



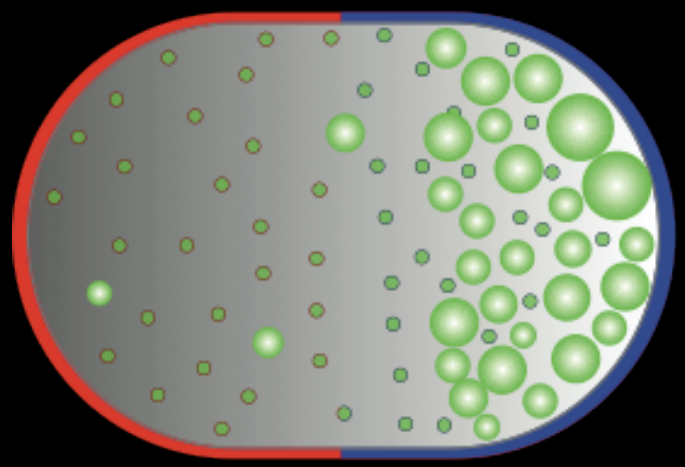
Position along cell axis

P granule assembly/disassembly changes with time

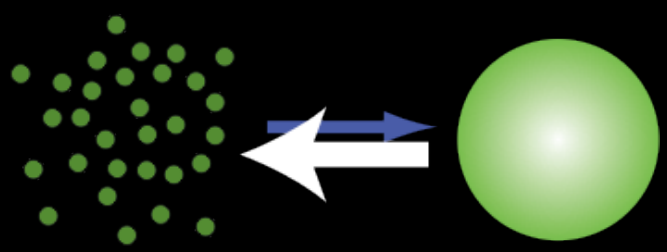


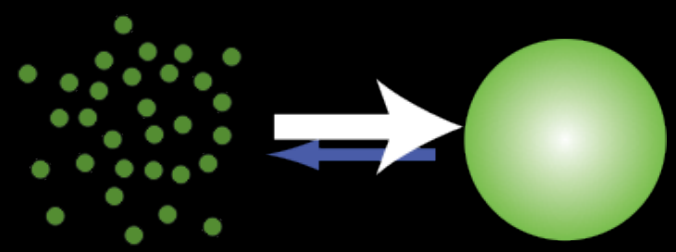
P granules separate by a gradient of assembly/dissasembly

Dissolution favored



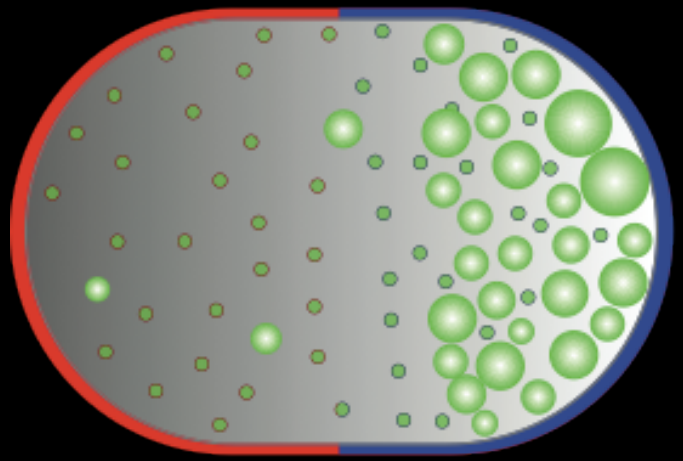
Formation favored





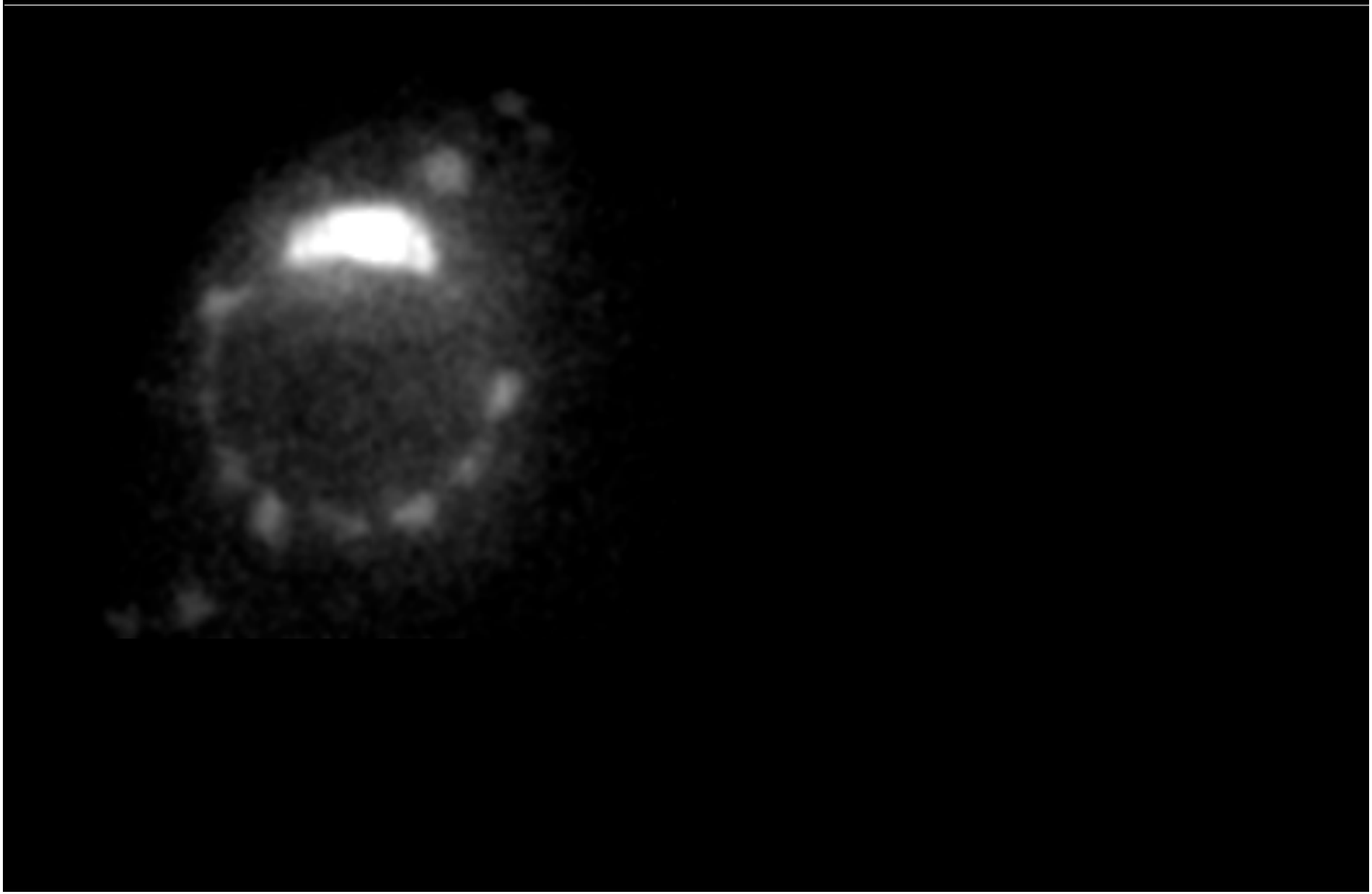
Why do P granules form at the posterior?

Dissolution favored



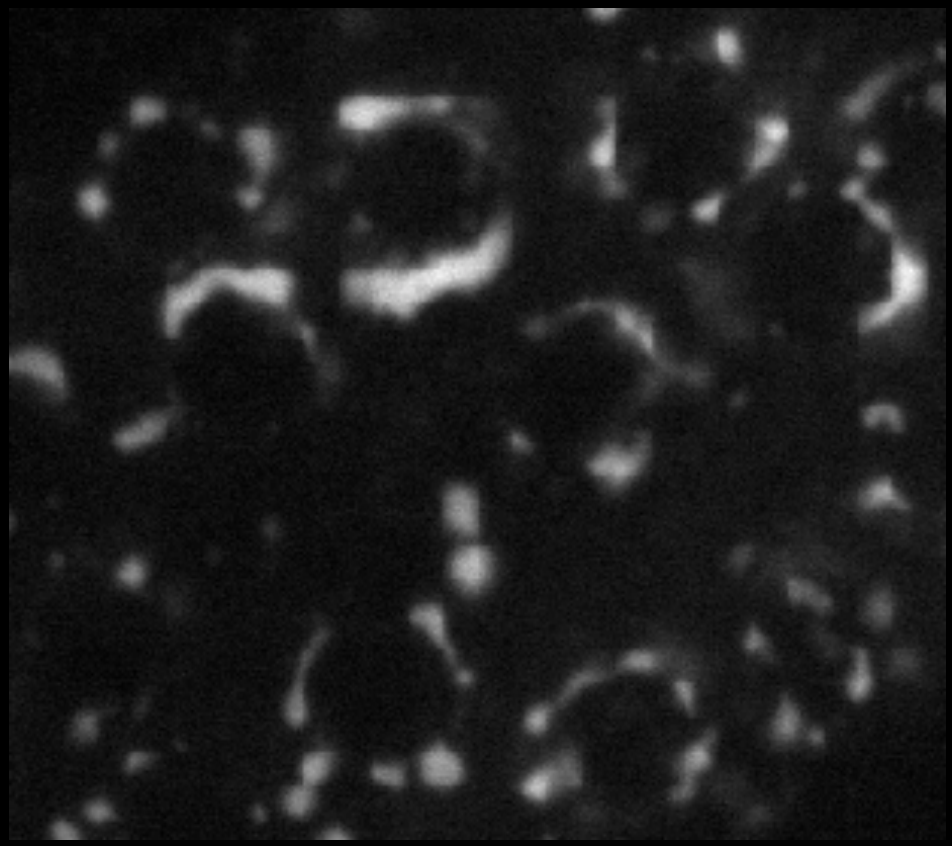
Formation favored

P granules fuse like liquids drops



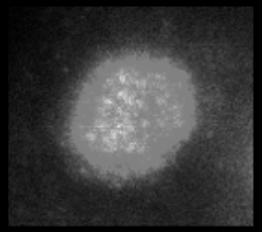
P granules fuse like liquids drops

P granules drip



P granules behave like liquid droplets

P granules are spherical

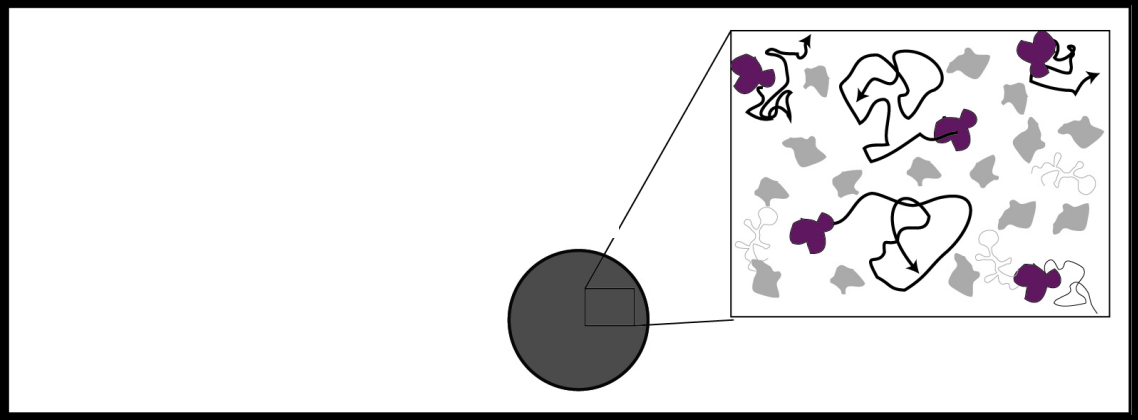


P granules wet when attached to the nucleus

P granules can fuse into larger spheres

Nuclear associated
P granules undergoing fusion

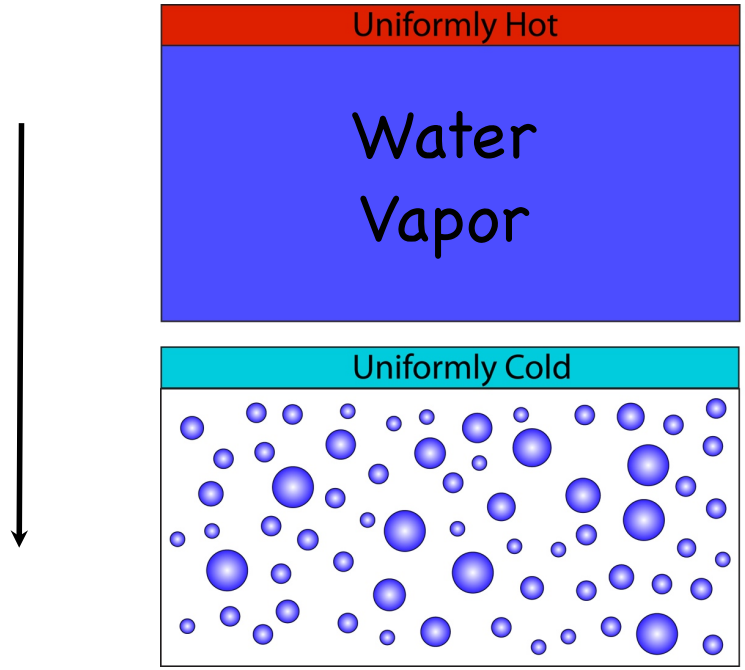
Liquids have rapid turnover of internal contents



Liquids undergo phase transitions

Liquids undergo phase transitions

Decrease Temperature



At lower temperatures the vapor is saturated, and condenses into water droplets

P granules formation can be viewed as a liquid-liquid phase transition

dilute phase condensed phase

Phase transitions are a simple way of concentrating complex mixtures of reactants

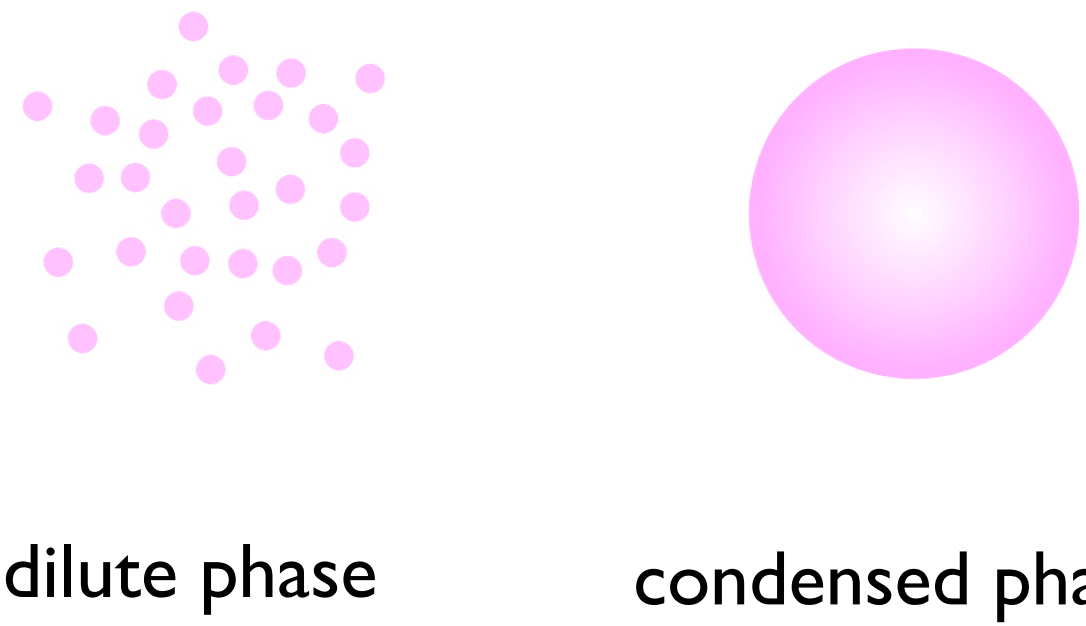
Phase transitions are a simple way of concentrating complex mixtures of reactants

Simple rules can lead to large scale reorganization

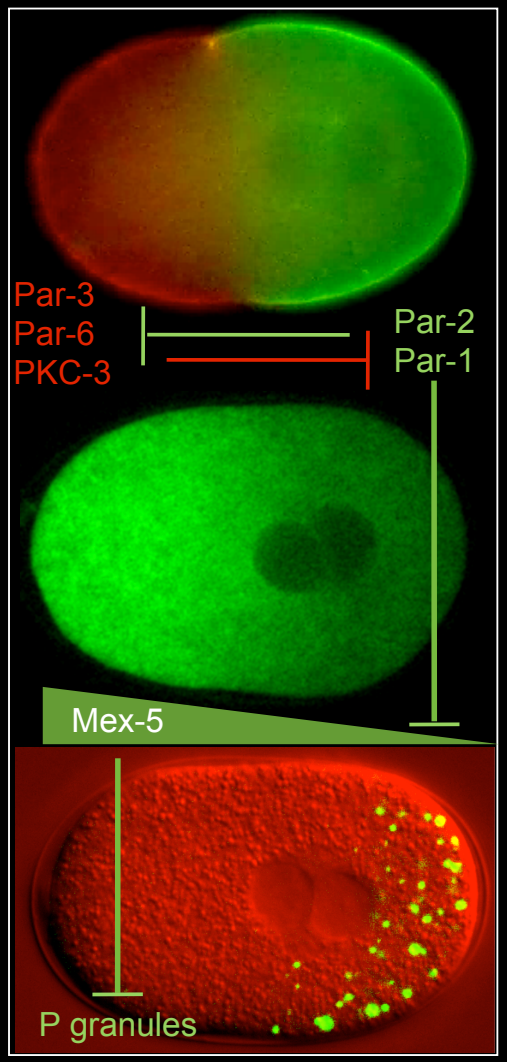
Phase transitions are a simple way of concentrating complex mixtures of reactants

In the future it will be essential to describe these rules at a molecular level.

Why do P granules condense at the posterior?



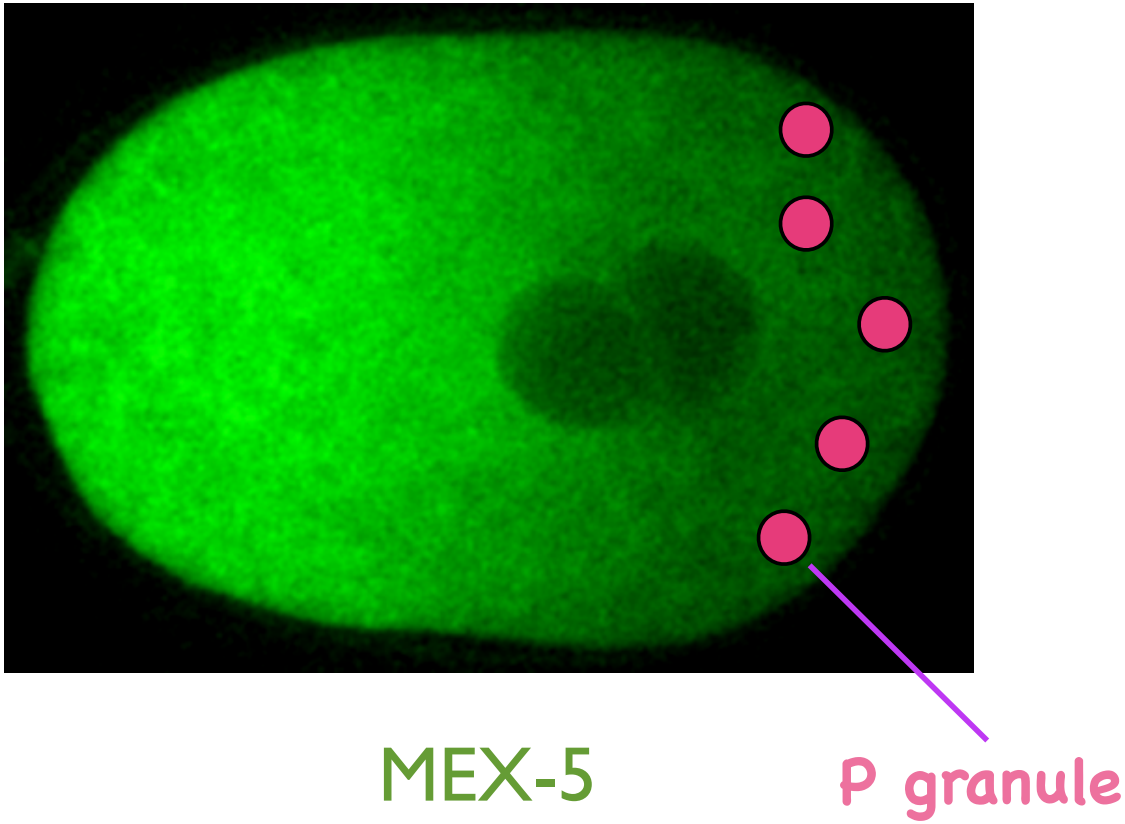
Underlying biochemical asymmetries in polarity



Watts, Priess, Kemphues et.al. 1996
Goehring, Hyman et.al. 2010

Tenlen et.al. 2008
Schubert, Priess et.al. 2000

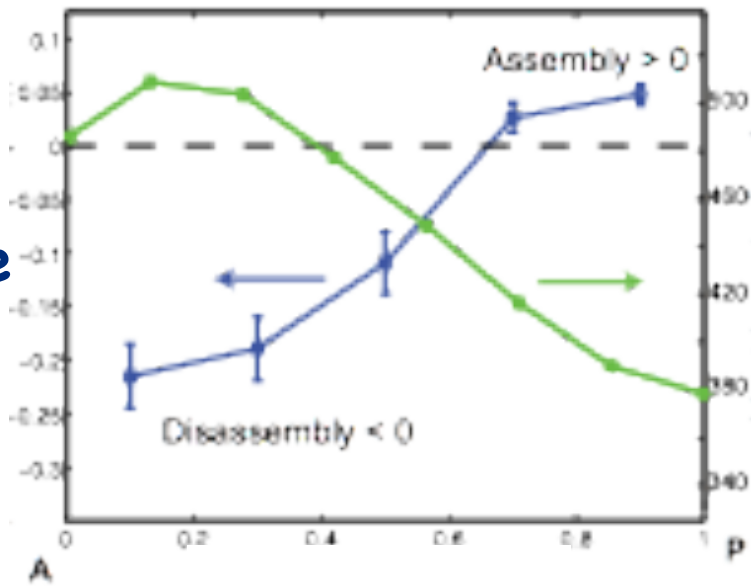
Mex-5 establishes a complementary gradient to P granule assembly



Brangwynne et al 2010

Mex-5 establishes a complementary gradient to P granule assembly

P Granule Intensity

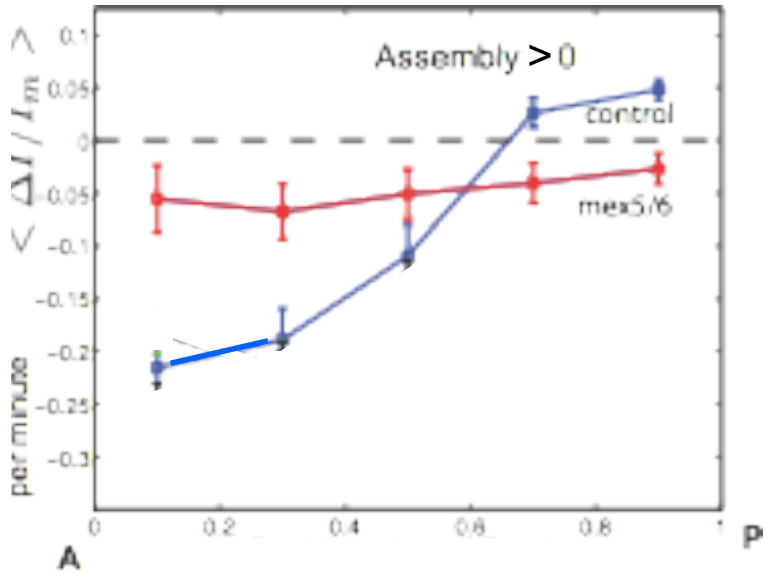


Mex 5 Intensity

Position along cell axis

Mex-5 is required for P granule assembly

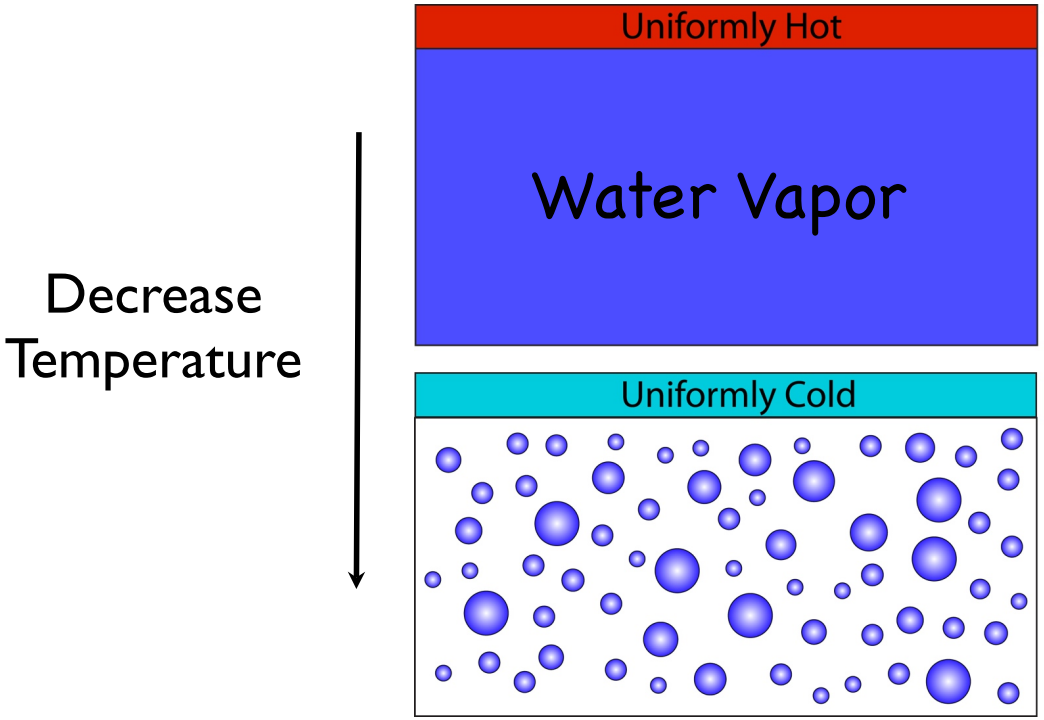
P Granule
Intensity
Wildtype



P Granule
Intensity
Mex5
mutants

Position along
cell axis

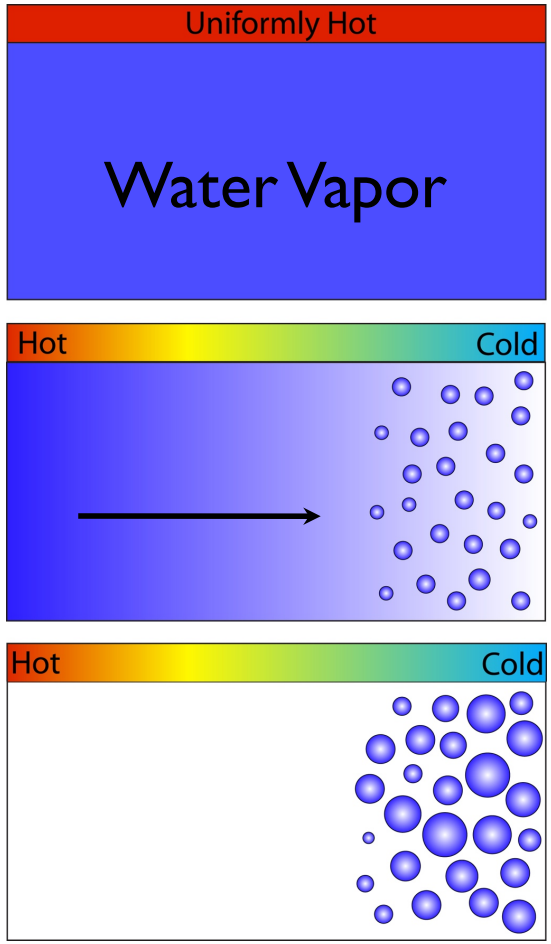
Water Vapor condenses into Droplets



At lower temperatures the vapor is saturated, and condenses into water droplets

Imposing a temperature gradient segregates water

diffusive flux of water vapor



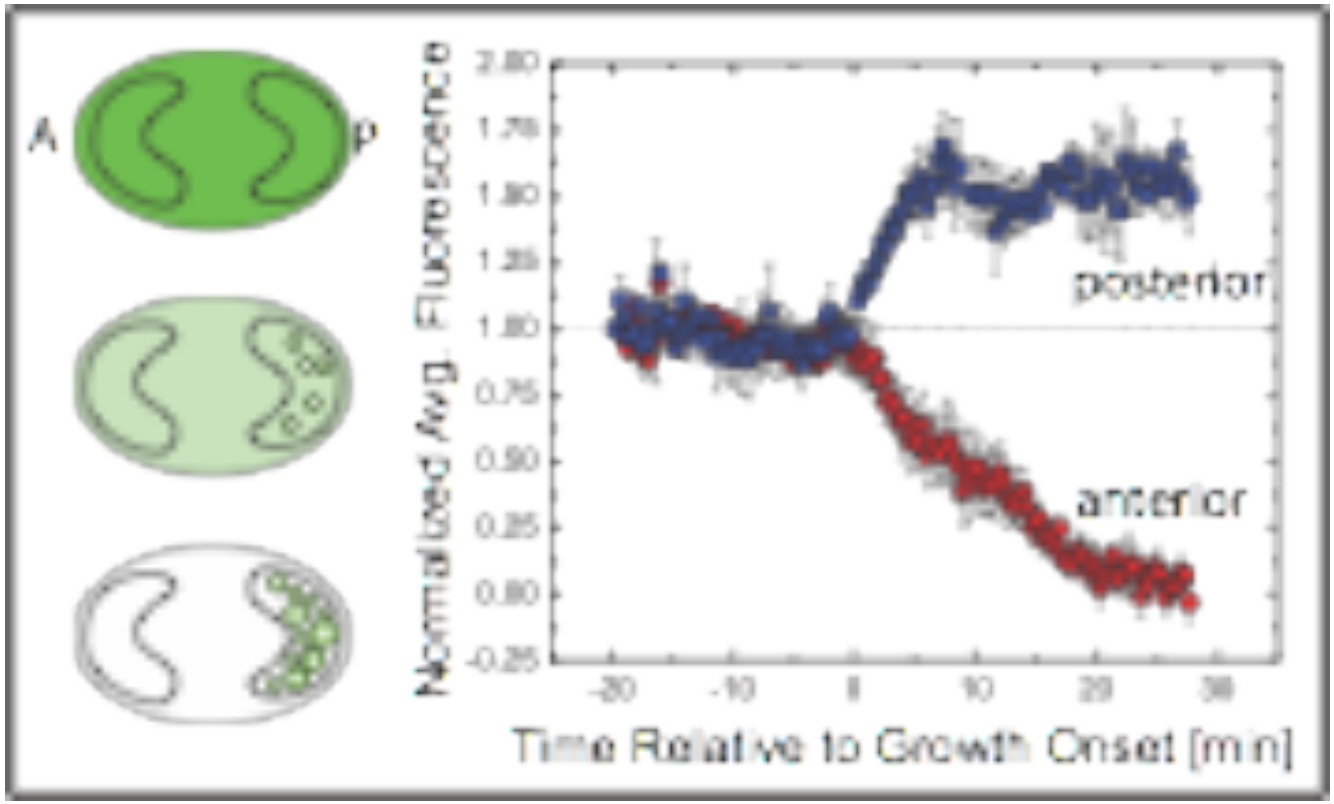
Water is condensing at the end of the vessel

This is what we believe happens when P granules condense.

BUT.... the gradient in the saturation point is not established by a temperature gradient.

Rather the saturation point is established by a gradient in polarity proteins, such as MEX-5

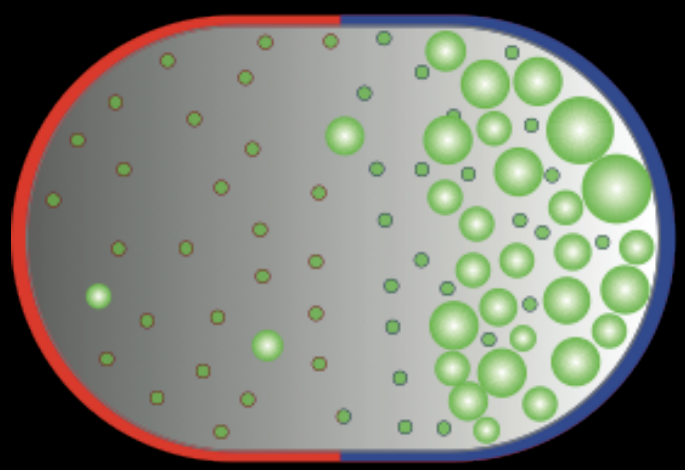
Diffusive flux drives P granule assembly



GFP::PGL-1

Why do P granules form at the posterior?

P granules segregate because the diffusion rate of the liquid phase is much slower than the diffusion rate of the dissolved phase



E.B. Wilson 1899

Cytoplasm is a colloidal liquid emulsion

SCIENCE

EDITORIAL COMMITTEE: S. NEWCOMB, Mathematics; R. S. WOODWARD, Mechanics; E. C. PICKERING, Astronomy; T. C. MENDENHALL, Physics; R. H. THURSTON, Engineering; IRA REMSEN, Chemistry; J. LE CONTE, Geology; W. M. DAVIS, Physiography; HENRY F. OSBORN, Paleontology; W. K. BROOKS, C. HART MERRIAM, Zoology; S. H. SCUDDER, Entomology; C. E. BESSEY, N. L. BRITTON, Botany; C. S. MINOT, Embryology, Histology; H. P. BOWDITCH, Physiology; J. S. BILLINGS, Hygiene; J. MCKEEN CATTELL, Psychology; DANIEL G. BRINTON, J. W. POWELL, Anthropology.

FRIDAY, JULY 14, 1899.

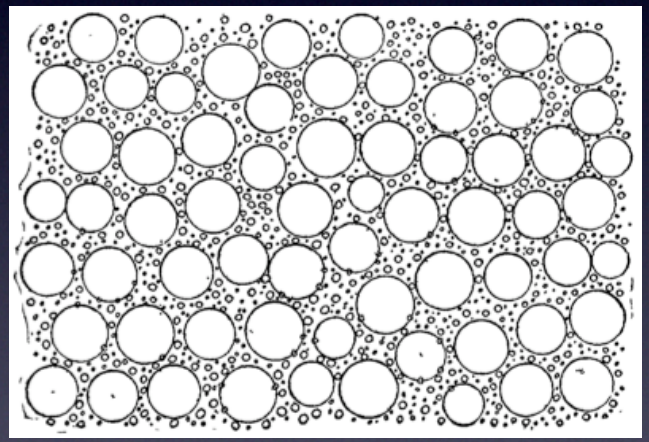
CONTENTS:

MSS. intended for publication and books, etc., intended for review should be sent to the responsible editor, Professor J. McKeen Cattell, Garrison-on-Hudson N. Y.

*THE STRUCTURE OF PROTOPLASM.**

IT would be superfluous to dwell in this place on the deep and enduring interest that attaches to the microscopical study of protoplasm. Since the time when the studies of Cohn and Schultze led to the general recognition of protoplasm as the material substratum of vital activity—a conclusion so eloquently set forth by Huxley in his celebrated essay on the physical basis of life—this interest has continually increased, as we have come to see even more clearly that all biological phenomena are directly or indirectly traceable to the effects of protoplasmic activity, for we have thus been impelled to seek for an understanding of that activity in the morphological structure of protoplasm, as revealed by the microscope. It is small wonder that to this quest some of the ablest of modern biologists have devoted their best energies. And yet, if we take account of the actual

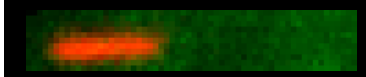
* This lecture is printed by permission of Professor C. O. Whitman, Director of the Biological Laboratory at Wood's Holl, and Messrs. Ginn & Co., the publishers of 'Biological Lectures delivered at the Marine Biological Laboratory, 1889–99,' in which it will appear. A more adequately illustrated special paper on the subject, containing more specific references to the literature, is now in press. It should be borne in mind that such delicate textures as those seen in the protoplasm of living cells cannot be properly illustrated by black and white figures. The accompanying text figures, though copied as accurately as possible from the original drawings, are of necessity relatively rude and schematic.

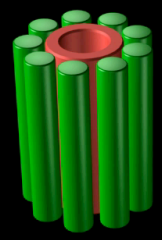


A "short" history of 20th century cell biology

1st half 20th Century	Physical chemistry of the cytoplasm -Think about the cytoplasm with no knowledge of molecules
2nd half 20th Century	Molecular biology -Catalogue and understand the molecules of the cell.

Length scale





Polymer

Virus-like

Liquids