Spatial Organization of the Cell Cytoplasm by Position-Dependent Phase Separation

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During asymmetric cell division, cytoplasmic components are segregated to opposite sides of the cell. We discuss how the observed segregation can be achieved by a position-dependent phase separation mechanism controlled by a protein concentration gradient. We show that effects of even a weak gradient can be amplified by the phase transition to achieve strong segregation. We compare our theory to the segregation of germ granules observed during the divisions in the *C. elegans* embryo. Our study demonstrates how liquid-liquid phase separation can play a key role in the organization of the cytoplasm.

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The cytoplasm of living cells is a complex fluid that exhibits spatial organization at different scales [1]. Its dynamical and mechanical properties play an important role in a number of biological processes, but remain poorly understood [2]. An intriguing example displaying the dynamic nature of the cytoplasm is observed during asymmetric cell division which involves the unequal distribution of cytoplasmic components to the two daughter cells. A well-studied case of this phenomenon is the asymmetric cell division of the fertilized egg of the *C. elegans* embryo [3] where germ granules in the cytoplasm localize preferentially to the posterior side and subsequently, end up in the posterior daughter cell. Germ granules, which in *C. elegans* are called P granules, are implicated in the specification of the germline of the organism. They are, therefore, found in precursor germ cells such as the posterior daughter cell after the first division of the egg [3,4]. P granules are non-membrane-bound assemblies of RNA and proteins in the cytoplasm that can be up to microns in size. It has been recently shown that P granules exhibit liquid droplet like properties, which suggests that they are a condensed liquid phase coexisting with the surrounding cytoplasm [5]. For *C. elegans*, P granule localization and asymmetric cell division, in general, is achieved with the help of the asymmetric compartmentalization of the cell membrane. After fertilization of the egg, two domains of different protein composition are set up in the cell membrane [see Fig. 1(a)] [6–8], which subsequently regulate the formation of graded cytoplasmic protein distributions, such as the Mex-5 gradient [Fig. 1(b)]. The formation of the Mex-5 protein gradient then guides the localization of P granules to the posterior side of the cell [Fig. 1(c)]. Before the Mex-5 concentration gradient is established along the anterior-posterior axis of the cell, P granules are distributed uniformly throughout the cytoplasm. As the Mex-5 gradient forms, P granules become localized to the posterior side by position-dependent assembly and disassembly. High Mex-5 concentration is associated with P granule disassembly, and a reduction of Mex-5 leads to a slow-down of P granule disassembly [5,6,9–11]. The P granule liquid properties and their localization behavior suggests that P granules form by phase separation from the cytoplasm [5]. These experimental observations have, thus, led to the proposal that P granule formation and localization results from a phase separation in the cytoplasm in which the supersaturation is controlled by Mex-5 [Fig. 1(a)] [5]. This raises the question how a weak concentration gradient of Mex-5 protein could be amplified by a phase separation to a sharply segregated distribution of P granules.

In this Letter, we provide a theoretical framework that accounts for P granule assembly, disassembly, and localization as resulting from the cytoplasmic Mex-5 concentration gradient. In our model, the Mex-5 gradient induces

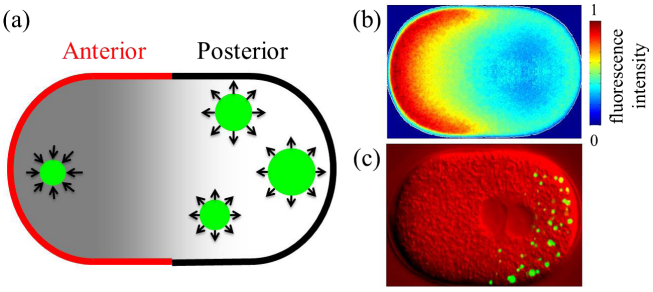

FIG. 1 (color online). (a) Schematic representation of the polarized *C. elegans* embryo at the one-cell stage prior to asymmetric cell division. Cell polarity is established by asymmetric localization of proteins in the anterior (red) and posterior (black) cell membranes. This asymmetry is required to generate a cytoplasmic gradient of Mex-5 protein (gray). P granules (green) segregate to the posterior side where they grow, while they disappear in the anterior side. (b) False color representation of fluorescence intensity of Mex-5 tagged with green fluorescence protein (GFP) averaged over four embryos reflecting the spatial distribution of Mex-5 proteins in the cell. (c) Image of the *C. elegans* embryo superimposed with the fluorescence image of PGL-1 tagged with GFP labeling P granules in the cell. Note that P granules are localized at the posterior side of the cell.

a position-dependent phase separation with supersaturation of P granule components in the posterior side while at the same time, these components are undersaturated in the anterior side. Starting from a thermodynamic description of a multicomponent system and assuming local equilibrium, our mechanism leads to an inhomogeneous state, which globally is not at equilibrium, with a fully mixed P granule-cytoplasm phase in the anterior side and phase separated P granule droplets coexisting with the cytoplasm in the posterior. Finally, we relate our model to observed P granule distributions in the *C. elegans* embryo.

We first consider the interactions between Mex-5 and P granules. Figure 1(b) shows a quantification of the Mex-5 concentration in the midplane of the *C. elegans* embryo. An example of the distribution of P granules is shown in Fig. 1(c) [5]. This figure shows that Mex-5 and P granules accumulate at opposite sides of the cell. Furthermore, it is known that a higher concentration of Mex-5 is observed inside the P granules as compared to the surrounding cytoplasm [12]. These observations, together with the finding that Mex-5 levels stimulate P granule disassembly, suggest that Mex-5 is involved in both the assembly and disassembly of P granule droplets. This seemingly contradictory role of Mex-5 can be understood by a simple physical scenario in which P granule constituents phase separate from the cytoplasm.

For simplicity, we describe the cytoplasm as a ternary fluid consisting of (i) P granule constituents with volume fraction ϕ, (ii) Mex-5 protein with volume fraction ψ, and (iii) cytoplasmic constituents with volume fraction θ, such that $\phi + \psi + \theta = 1$. This implies that only two volume fractions characterize the composition of the system. Assuming local equilibrium, a free energy density can be introduced. We use a simplified Flory-Huggins theory to describe this system and express the free energy density as [13]

$$f = k_B T[\phi \ln\phi + \psi \ln\psi + \theta \ln\theta] + \chi_{\phi\psi}\phi\psi + \chi_{\phi\theta}\phi\theta + \chi_{\psi\theta}\psi\theta. \quad (1)$$

Here, the first line of Eq. (1) describes entropic contributions, favoring mixing, and the second line accounts for effective interactions between the three components. Positive values of the coefficients χ_{ij} imply that mixing of the components i and j is unfavorable as it increases the free energy, while negative values imply that the free energy is reduced upon mixing components i and j [14]. That the P granule constituents can phase separate from cytoplasmic constituents implies that $\chi_{\phi\theta} > 0$. The observed colocalization of Mex-5 with both P granules and solvent correspond to the situation where $\chi_{\phi\psi} < 0$ and $\chi_{\psi\theta} < 0$ [12]. With this choice of interactions, the ternary system exhibits a phase diagram with a region of mixing and a region of two-phase coexistence. This phase diagram is shown in Fig. 2(a) for $\chi_{\phi\psi} = -5k_BT$, $\chi_{\phi\theta} = 3k_BT$, and $\chi_{\psi\theta} = -2k_BT$. At low Mex-5 volume fraction ψ, the system phase separates. This corresponds to the formation of P granule droplets in the cytoplasm. With increasing ψ, the coexistence region shrinks until it disappears beyond a threshold value. This implies that Mex-5, which mixes with both P granule and cytoplasmic components, can dissolve P granules in the cytoplasm. Furthermore, for $\chi_{\phi\psi} < \chi_{\psi\theta} < 0$, the lines connecting coexisting phases [broken lines in Fig. 2(a)] are tilted from the upper left side to the lower right side on the phase diagram. This corresponds to the case in which Mex-5 has a larger volume fraction inside the P granule phase than in the cytoplasmic phase, as is indeed observed [15].

The phase diagram of Fig. 2(b) can account for the effects of Mex-5. A Mex-5 gradient leads to the occurrence of P granule droplets in the posterior side and the dissolution of P granules in the anterior side. The two gray points (A and P) in the phase diagram corresponding to the anterior and posterior poles of the cells are indicated in Fig. 2(b). The connecting line between those points accounts for the change of Mex-5 levels ψ in the cell and the accumulation of overall P granule material in the posterior observed experimentally (see Fig. 3). The exact ψ and ϕ in the cell are currently unknown, and the positioning of the gray lines reflects our experimental observations that the Mex-5 concentration has a two-fold difference between the anterior and the posterior points

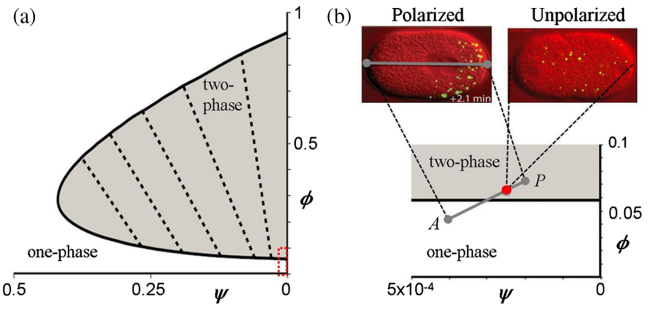

FIG. 2 (color online). (a) Phase diagram for a ternary fluid description of the cytoplasm, where ψ denotes the volume fraction of P granule constituents and ϕ denotes the volume fraction of the Mex-5 protein. In the one-phase region, all components mix. For small ψ, a two-phase coexistence region exists in which P granules coexist with the cytoplasm. The broken lines connect coexisting phases. The region encapsulated by the red broken line corresponds to the region of the phase diagram shown in (b). (b) The phase diagram at low ϕ and low ψ limits. The gray tiled line indicates schematically a range of Mex-5 concentrations corresponding to a gradient spanning from anterior (A) to posterior (P) in a polarized cell. Note that P granule segregation implies that (A) is in the one-phase region while (P) is inside the two-phase region. The case of a non-polarized cell with P granules in the cytoplasm corresponds to a single point (red) in the two-phase region in the phase diagram. Parameter values of the phase diagram shown are $\chi_{\phi\psi} = -5k_BT$, $\chi_{\phi\theta} = 3k_BT$, and $\chi_{\psi\theta} = -2k_BT$.

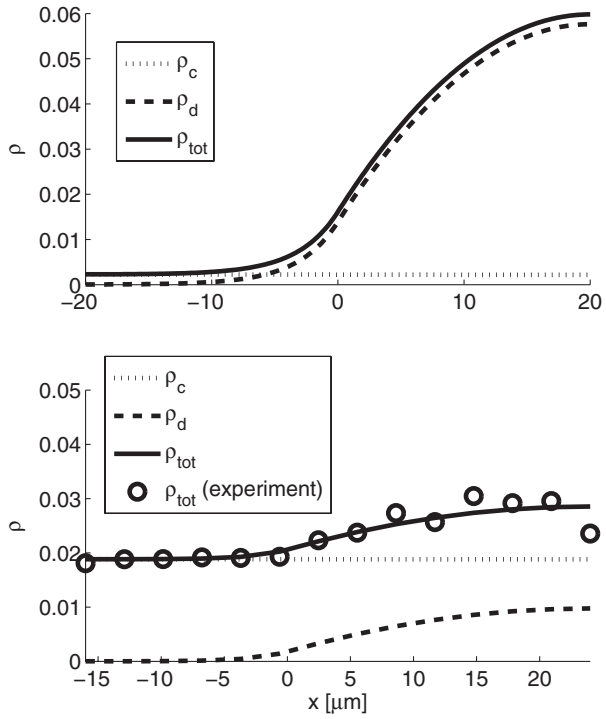

FIG. 3. (Upper figure) Calculated steady-state distribution of the total P granule mass density $\rho_{\mathrm{tot}}(x) = \rho_d(x) + \rho_c(x)$ solid line), the mass density of the droplet form $\rho_d(x)$ (dash line), and of the cytoplasmic form $\rho_c(x)$ (dotted line) as a function of position x. Droplets segregate to the region $x > 0$ where phase separation occurs, while they dissolve in the region $x < 0$. Parameter values are $D_c = 10\ \mu\mathrm{m}^2/\mathrm{s}$, $D_d = 0.01\ \mu\mathrm{m}^2/\mathrm{s}$, $\alpha = \beta = 10^{-3}/\mathrm{s}$, and $L_1 = L_2 = 20\ \mu\mathrm{m}$. The y axis is rescaled so that the total mass $\int dx \rho_{\mathrm{tot}}(x) = 1$. (Lower figure) Comparison of the theory with experimental data. The experimentally observed distribution $\rho_{\mathrm{tot}}(x)$ of P granule constituents in a polarized *C. elegans* embryo is shown (black circles). It is obtained from the fluorescence intensity of PGL-1 tagged with GFP. Note that data close to the cell poles are omitted due to boundary effects. As a result, the total length of the system is 40 μm. A comparison of the steady-state solution of Eq. (4) to the experimental data is shown by the solid line. For the same solution, the mass densities for the droplet form ρ_d (dash line) and for the cytoplasmic form ρ_c (dotted line) are also shown. Values for the diffusion constants were chosen as are $D_c = 10\ \mu\mathrm{m}^2/\mathrm{s}$, $D_d = 0.01\ \mu\mathrm{m}^2/\mathrm{s}$. The fitted parameter values are $\alpha = 1.5 \times 10^{-5}/\mathrm{s}$, $\beta = 1.4 \times 10^{-3}/\mathrm{s}$, $L_1 = 24\ \mu\mathrm{m}$, and $L_2 = 16\ \mu\mathrm{m}$. The y axis is rescaled so that the total mass $\int dx \rho_{\mathrm{tot}}(x) = 1$.

[Fig. 1(b)] while the concentration of P granule material is about 1.5 times higher at the posterior point than at the anterior point [Fig. 3(b)].

Since the line crosses the phase boundary, we have a situation where P granule droplets coexist with the cytoplasm in the posterior, while P granules dissolve in the anterior side. An unpolarized cell has no Mex-5 gradient and thus, corresponds to a single point in the phase diagram as indicated by the red dot in Fig. 2(b). We, thus, propose a scenario where the Mex-5 gradient controls phase separation of P granule constituents and cytoplasm such that two-phase coexistence occurs only in the posterior side while in the anterior side, all the components (Mex-5, P granule constituents, and cytoplasmic constituents) mix.

So far, we have employed local equilibrium arguments to characterize P granule behavior. However, it is important to emphasize that the system as a whole is dynamic and not at equilibrium. The system is driven out of thermodynamic equilibrium by the processes which maintain the Mex-5 concentration gradient. This gradient is generated by phosphorylation and dephosphorylation of Mex-5 under the guidance of asymmetric membrane domains [16–18]. Thus, a nonequilibrium description of the ternary fluid is required to understand the distribution of P granule constituents in the presence of a Mex-5 gradient.

The position-dependent phase behavior of the system represents a dynamic state in which P granule droplets that form in the posterior can diffuse to the anterior side where they can subsequently dissolve. At the same time, P granule constituents that mix with the cytoplasm on the anterior side can diffuse to the posterior where they either feed the growth of P granule droplets or promote the nucleations of new droplets. The dynamics can be captured in a simplified form as follows: we describe the dynamics of P granule droplets by a diffusion equation for the mass density $\rho_d(x)$ of P granule droplets at position x in the cell. Source and sink terms account for droplet formation, growth, and dissolution. For simplicity, we assume that all droplets grow rapidly to a typical size with droplet mass m_d and that all droplets have this mass. In this case, the distributions of droplets obeys

$$\partial_t \rho_d = D_d \nabla^2 \rho_d - k^-(x, \rho_c)\rho_d + m_d k^+(x, \rho_c), \quad (2)$$

where D_d is the diffusion constant of the droplets, the rate of droplet formation per unit volume is denoted by k^+, and the rate of disappearance of droplets is denoted by k^-. In general, both rates depend on the local mass densities of dissolved P granule constituents in the cytoplasm, denoted by ρ_c. In addition, both rates depend on the local Mex-5 concentration. Since we assume that the Mex-5 concentration gradient is imposed, we describe the effects of the Mex-5 protein by the position dependence of the rates k^+ and k^-.

Similarly, we write an equation that describes the mass density ρ_c of the dissolved P granule constituents:

$$\partial_t \rho_c = D_c \nabla^2 \rho_c - m_d k^+(x, \rho_c) + k^-(x, \rho_c)\rho_d. \quad (3)$$

Here, D_c is the diffusion coefficient for the cytoplasmic P granule constituents, and the source and sink terms are chosen such that total mass density of P granule constituents $\int dx \rho_{\mathrm{tot}}(x) = \int dx[\rho_d(x) + \rho_c(x)]$ is conserved.

To illustrate the basic properties of this system, we focus on the steady-state solution of Eqs. (2) and (3). We consider a simplified one-dimensional system in which

droplets nucleate and grow on the posterior for $x > 0$ and disappear on the anterior side for $x < 0$. The droplet production rate is assumed to be proportional to the mass density of the cytoplasmic P granule constituents ρ_c. We, therefore, choose $m_d k^+(x, \rho_c) = \alpha\Theta(x)\rho_c$ and $k_-(x, \rho_c) = \beta\Theta(-x)$, where the Heaviside step function $\Theta(x)$ describes the effect of the phase boundary in Fig. 2, and the parameters α and β are effective rates of conversion between droplets and cytoplasmic components. Note that the Heaviside function employed here reflects the highly nonlinear and cooperative nature of phase transition. The expressions for k^+ and k^-, thus, capture the situation where the cytoplasm is partitioned into two regions such that a one-phase region exists for $L_2 \leq x < 0$ (the high Mex-5 concentration region) while phase separation occurs for $0 \leq x \leq L_1$ (the low Mex-5 concentration region). Note that $L_1 + L_2$ is the length of the system.

The steady-state solution to Eqs. (2) and (3) with no-flux boundary conditions at $x = -L_2$ and $x = L_1$ is

$$\rho_c(x) = \begin{cases} A\cosh[\gamma_1(L_1 - x)], & x \geq 0 \\ B - C\cosh[\gamma_2(L_2 + x)], & x < 0 \end{cases}$$
$$\rho_d(x) = \frac{D_c}{D_d}[B - \rho_c(x)]. \tag{4}$$

In the above equation, A is an arbitrary constant that sets the overall mass of P granule constituents in the system, and

$$B = A\cosh(\gamma_1 L_1)(1 + \gamma_1/\gamma_2), \tag{5}$$

$$C = \frac{A\gamma_1\cosh(\gamma_1 L_1)}{\gamma_2\cosh(\gamma_2 L_2)}. \tag{6}$$

Furthermore, $\gamma_1^2 = \alpha/D_d$, $\gamma_2^2 = \beta/D_c$ An example of such a solution is shown in Fig. 3(a) demonstrating the possibility for strong segregation and localization using position-dependent phase separation. The solid line shows the overall density profile $\rho_{\text{tot}}(x)$ with pronounced accumulation in the posterior side. This accumulation is a consequence of droplet localization on the same side (dash line). The soluble constituents also show a concentration profile (dotted line), albeit with a much weaker gradient by comparison.

We can now relate the steady state solution [Eq. (4)] to the observed segregation of P granules during cell division. The diffusion coefficient of cytoplasmic Pgl-1, a major constituent of P granules, may be estimated as $D_c \simeq 10\ \mu\text{m}^2/\text{s}$ due to its size [5]. The diffusion coefficient D_d of droplets can again be estimated from previous tracking data of diffusing objects of similar size in the cytoplasm, which gives $D_d \simeq 0.01\ \mu\text{m}^2/\text{s}$ [5]. Note that $D_c \gg D_d$, which is expected because of the size difference between freely diffusing P granule constituents and P granule droplets.

Using the fluorescently labeled P granule component PGL-1, we can quantify the density profile of this component in both droplets and cytoplasm as a function of position (see the circles in Fig. 3). By comparing Eq. (4) to this data, we can estimate the unknown conversion rates α, β, L_1, and L_2. We find that the P granule assembly rate α is 2 orders of magnitude smaller than the disassembly rate β, and that $L_1 > L_2$, which suggests that the phase boundary is located to the anterior side from the midpoint of the cell. For these parameters, our model predicts that P granule droplets exist predominantly in the posterior (as indicated by the dotted line), which is consistent with experimental observations as described before (see Figs. 1 and 2).

In summary, we have theoretically analyzed a system in which a gradient of supersaturation is maintained. We show, using a simplified theoretical description of droplet dynamics, that a weak chemical gradient can be strongly amplified by the phase transition which acts as a switch-like element. In a supersaturation gradient, droplets undergo cycles of growth and disassembly, leading to a nonequilibrium steady state in which components can be segregated and localized. This scenario is relevant for the localization of P granule droplets to one side of the cell during asymmetric cell division in the *C. elegans* embryo. In this example, the principle of using phase separation for segregation implies that a weak concentration gradient of Mex-5 could induce a pronounced accumulation of P granule material on one side of the cell. Viewing the cytoplasm as an emulsion, the system is stabilized by nonequilibrium conditions under which coarsening is prevented and droplets constantly turn over. In addition to P granules, other liquid phase droplets have been identified in cells, including nucleoli [19] while P bodies, Cajal bodies, stress granules, and other non-membrane-bound intracellular assemblies also appear to represent liquid phase droplets [20,21]. We, therefore, anticipate that liquid-liquid phase separation of the cytoplasm may be an important principle for the spatial organization of the cell. Position-dependent phase separation provides a powerful tool for patterning the cell using weakly graded cues. This is reminiscent of morphogen gradients in tissues, where nonlinear signaling processes turn weak gradients into position-dependent downstream effects and patterns. Here, we propose that in the cell similar transduction of concentration gradients can occur via physical mechanisms involving a phase transition in the cytoplasm that provides nonlinear amplification and switch-like responses.

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