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Phase separation driven by production of architectural RNA transcripts†

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We here use an extension of the Flory-Huggins theory to predict that the phase separation is driven by the production of architectural RNA (arcRNA) at a DNA locus with a constant rate. The arcRNA molecules diffuse in the nucleoplasm and show attractive interactions via proteins that are bound to the arcRNA. Our theory predicts that when the Flory interaction parameter is larger than the value at the critical point, the volume fraction of arcRNA jumps between the two values, corresponding to the volume fraction of the two coexisting phases in the equilibrium, at a distance from the DNA locus due to the local equilibrium condition. The distance defines the radius of the condensate that is assembled by the phase separation. When the interaction parameter is large, the volume of the condensates is proportional to the production rate of arcRNA and inversely proportional to the degradation rate of arcRNA. These results imply that most arcRNA molecules are degraded before it diffuses out from the condensates due to the strong segregation of arcRNA.

1 Introduction

Cell nucleus is not a uniform solution of chromatin and proteins, but shows a number of nuclear bodies, such as the nucleoli, paraspeckles, and Cajal bodies[1]. These nuclear bodies are the condensates of proteins and/or RNA. It has recently found that transcription factors (such as OCT4) and cofactors (such as the Mediator complexes) form condensates that colocalize with superenhancers and these condensates play an important role in gene expression[2–4]. There are growing evidences that these nuclear bodies form due to the phase separation and the non-specific interactions between the intrinsically disordered domains (or prion-like domains) of constituent proteins play an important role in driving the phase separation[5–7].

The architecture of paraspeckles is composed of long non-coding architectural RNA molecules (arcRNA[1]), which are glued by multiple paraspeckle proteins[8–11]. These condensates are usually observed at the proximity of the DNA loci, at which arcRNA is produced, and are disassembled when the transcription is suppressed[12]. Transcriptional condensates show similar features[3,4]. The dynamics of the arcRNA production by transcription plays an important role in the formation of the condensates, probably because the local concentration of the arcRNA is high at the vicinity of the DNA loci and/or due to the intermolecular RNA-RNA interactions during transcription[13]. One cannot therefore treat the condensates by using the classical theories that treat the phase separation in the equilibrium[14]. At the first glance, one may think that the formation of the condensates may be analogous to the fact that the concentration gradient of RNA polymerase is produced at the vicinity of active genes due to the uni-directional transport of the enzymes during the transcription[15,16]. However, in contrast to this case, arcRNA show attractive interactions via the binding proteins and these interactions also contribute to the formation of condensates.

We here use an extension of the Flory-Huggins theory to predict the phase separation driven by the production of arcRNA. With our treatment, the magnitudes of the interactions between arcRNA via the binding proteins are expressed by using the Flory interaction parameter. The polymerization of nucleoside triphosphate monomers to arcRNA drives the phase separation because the monomers of each arcRNA are already at the close vicinity to each other (as predicted by the Flory-Huggins theory). Our theory predicts that the condensates are induced as long as the interaction parameter is larger than the critical value. The polymer volume fraction jumps at the interface between the condensates and the nucleoplasm because the polymer volume fractions between those of two coexisting domains in the equilibrium are

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unstable. The radius of the condensates scales as one third of the production rate of arcRNA when the interaction parameter is large enough. This result implies that arcRNA produced by a DNA locus is degraded before it diffuses out to the exterior. We anticipate that our theory is the first step towards the understanding of the physical mechanism involved in the formation of nuclear bodies that are produced by the phase separation.

2 Model

We treat a region of nucleoplasm at the proximity of a DNA locus, at which arcRNA transcripts are produced with a constant rate. The arcRNA molecules are composed of N segments, each acts as a binding site of the binding proteins. When the chain segments are occupied by the binding proteins, these segments show attractive interactions. With this model, the free energy density f has the form

$$\begin{aligned}\beta f(\phi,\sigma) &= \frac{1}{N}\phi\log\phi+(1-\phi)\log(1-\phi)-\chi\sigma^2\phi^2 \\ &\quad +[\sigma\log\sigma+(1-\sigma)\log(1-\sigma)+\beta\varepsilon\sigma]\phi, \quad (1)\end{aligned}$$

where it should be minimized by the local volume fraction ϕ of arcRNA molecules and the local occupancy σ of the binding proteins. The first and second terms of eq. (1) are the free energy contribution of the translational entropy of arcRNA molecules and water molecules in the nucleoplasm. The third term of eq. (1) is the free energy contribution due to the attractive interaction between arcRNA molecules due to the binding of proteins and χ is the Flory interaction parameter that accounts for the interaction. The fourth term of eq. (1) is the free energy contribution due to the binding of proteins. The first and second terms in the square bracket is the free energy contributions due to the entropy with the binding state of the proteins and the third term is the free energy increase ε due to the binding of proteins*. β $(=1/(k_{\rm B}T))$ is the inverse of the thermal energy. The form of eq. (1) is analogous to the free energy used to treat the phase separation of liquid crystals due to the orientational ordering [17], of multi-component lipid membranes due to the chain conformational ordering [18–21], and of chromatin due to the assembly and disassembly of nucleosomes [22–24]. Recently, the discontinuous conformational transition of chromatin due to the switching of histone mark has been predicted by minimizing a similar free energy [25].

The dynamics of arcRNA is treated by using the time evolution equation

$$\frac{\partial}{\partial t}\phi(\mathbf{r},t)=k_{\rm p}Nv_0\delta(\mathbf{r})-k_{\rm d}\phi(\mathbf{r},t)-Nv_0\nabla\cdot\mathbf{J}(\mathbf{r},t). \quad (2)$$

The first term in the right side of eq. (2) is the production rate of arcRNA at the DNA locus, located at $\mathbf{r}=\mathbf{0}$ (shown by the Dirac's delta function $\delta(\mathbf{r})$), and $k_{\rm p}$ is the rate constant that accounts for this process (the volume fraction of arcRNA increases by Nv_0 when one arcRNA is produced, where v_0 is the volume of a segment). The second term is the rate of the degradation of arcRNA and $k_{\rm d}$ is the rate constant that accounts for this process. The third term represents the accumulation of arcRNA due to the diffusion. The flux $\mathbf{J}(\mathbf{r},t)$ of arcRNA has the form

$$\mathbf{J}(\mathbf{r},t)=-\frac{1}{\zeta}\frac{\phi(\mathbf{r},t)}{Nv_0}\nabla\left(\frac{\partial f}{\partial\phi}\right), \quad (3)$$

where ζ is the friction constant per segment. We assume that the binding of proteins is fast and thus the occupancy σ is determined by the local equilibrium condition

$$\log\frac{\sigma}{1-\sigma}+\beta\varepsilon-2\chi\sigma\phi=0. \quad (4)$$

Eq. (4) is derived by using $\frac{\partial f}{\partial\sigma}=0$.

3 Equilibrium phase diagram

We first analyze the phase separation in the equilibrium by minimizing the free energy

$$\begin{aligned}F &= f(\phi_1,\sigma_1)\frac{V_1}{v_0}+f(\phi_2,\sigma_2)\frac{V_2}{v_0} \\ &\quad -\mu(\phi_1\frac{V_1}{v_0}+\phi_2\frac{V_2}{v_0})+\Pi_{\rm osm}(V_1+V_2). \quad (5)\end{aligned}$$

The subscripts 1 and 2 indicates the quantities in coexisting phases 1 and 2. The first term is the free energy of phase 1 and the second term is the free energy of phase 2, eq. (1). The third and fourth terms are the Lagrange multiplier to conserve the number of arcRNA transcripts and the volume of the system. ϕ_1 and ϕ_2 are the volume fraction of arcRNAs in the phase 1 and 2. σ_1 and σ_2 are the occupancies of binding proteins in phases 1 and 2. V_1 and V_2 are the volume of phase 1 and 2. The minimization of the free energy F with respect to ϕ_1, ϕ_2, V_1, and V_2 lead to the fact that the chemical potential μ and the osmotic pressure $\Pi_{\rm osm}$ are both equal between the two coexisting phases [14],

$$\mu = \frac{\partial f(\phi_1,\sigma_1)}{\partial\phi_1}=\frac{\partial f(\phi_2,\sigma_2)}{\partial\phi_2} \quad (6)$$

$$\Pi_{\rm osm} = \frac{1}{v_0}\phi_1^2\frac{\partial}{\partial\phi_1}\left(\frac{f(\phi_1,\sigma_1)}{\phi_1}\right)=\frac{1}{v_0}\phi_2^2\frac{\partial}{\partial\phi_2}\left(\frac{f(\phi_2,\sigma_2)}{\phi_2}\right) \quad (7)$$

For $\beta\varepsilon>2$, eq. (4) has two stable solutions and one unstable solution of the protein occupancy σ for a range of the interaction parameter χ and the volume fraction ϕ. There is thus a first order phase transition at $\phi=1$ and $\chi=\chi_{\rm th}$, where the value of the protein occupancy jumps, see the green dot in fig. 1a and 2a. The occupancy of proteins in isolated arcRNAs is rather small because the binding energy is positive, but proteins can bind arcRNAs cooperatively when these transcripts are concentrated because of the attractive interactions between protein-bound arcRNAs. For larger values of the interaction parameter χ, the phase of large volume fraction ϕ and protein occupancy σ coexists with the phase of smaller volume fraction ϕ and protein occupancy σ, see figs. 1 and 2. It is the phase separation associated with the first order phase transition: the solvent molecules suppress the attrac-

* **More precisely, it is the binding energy relative to the chemical potential of binding proteins in the unbound state.**

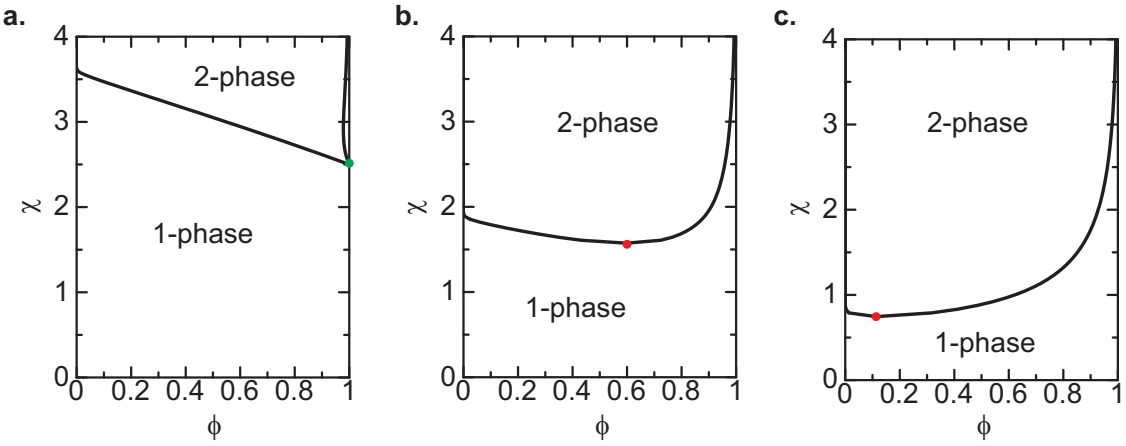

Fig. 1 Miscibility phase diagram is shown as a function of the volume fraction ϕ of arcRNA and the Flory interaction parameter χ for $\beta\varepsilon = 2.5$ (**a**), 0.5 (**b**), and -2.0 (**c**). We used $N = 100$ for the calculations. The first order phase transition point is shown by the green dot and the critical points are shown by the red dots.

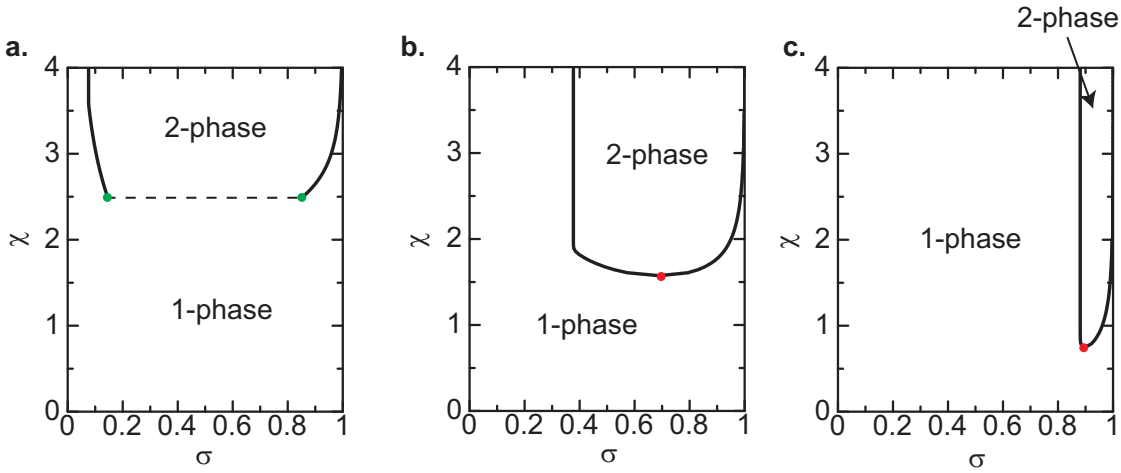

Fig. 2 Miscibility phase diagram is shown as a function of the occupancy σ of binding proteins and the Flory interaction parameter χ for $\beta\varepsilon = 2.5$ (**a**), 0.5 (**b**), and -2.0 (**c**). We used $N = 100$ for the calculations (these values correspond to fig. 1). The first order phase transition point is shown by the green dot and the critical points are shown by the red dots.

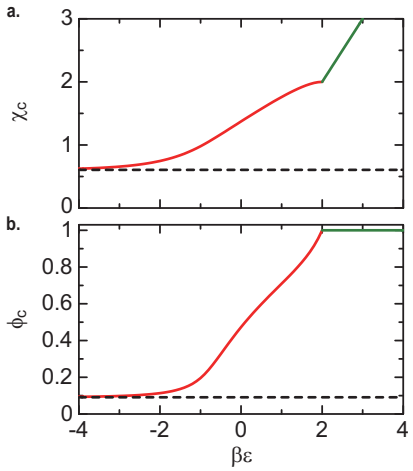

Fig. 3 The interaction parameter χ_c at the critical point (shown by the red curve) and the transition point (shown by the green curve) is shown as a function of the binding free energy $\beta\varepsilon$ (**a**). The polymer volume fraction at the critical point (shown by the red curve) and the transition point (shown by the green curve) is shown as functions of the binding free energy $\beta\varepsilon$ (**b**).

tive interactions between protein bound arcRNAs and thus are excluded from the region at which arcRNAs are concentrated to enhance the cooperative protein binding. For $\beta\varepsilon < 2$, eq. (4) has only one solution. In such cases, there is a critical point at the interaction parameter χ_c and the volume fraction ϕ_c, where two phases coexit for larger values of the interaction parameter, see figs. 1**b** and **c**. When the binding free energy $\beta\varepsilon$ is not very small, the volume fraction ϕ and the protein occupancy σ are relatively large in one phase and the volume fraction ϕ and the protein occupancy σ are smaller in the other phase. Both the critical interaction parameter χ_c and the critical volume fraction ϕ_c decrease with decreasing the binding free energy $\beta\varepsilon$, see fig. 3. The critical interaction parameter χ_c and the critical volume fraction ϕ_c reduce to the Flory-Huggins results

$$\chi_{\rm fh} = \frac{(1+\sqrt{N})^2}{2N} \tag{8}$$

$$\phi_{\rm fh} = \frac{1}{1+\sqrt{N}} \tag{9}$$

for $\beta\varepsilon \to -\infty$.

4 Phase separation driven by transcription dynamics

We here treat the case in which arcRNA is produced at the DNA locus by transcription. We solve eq. (2) for the steady state $\partial\phi(\mathbf{r},t)/\partial t = 0$ with the boundary condition that the volume fraction $\phi(\mathbf{r},t)$ is zero for $r \to \infty$. r is the distance from the DNA locus. The distance by which arcRNA diffuses before it is degraded is $\lambda_d/\sqrt{N}$, where the diffusion length λ_d has the form

$$\lambda_d = \sqrt{\frac{k_B T}{\zeta k_d}}. \tag{10}$$

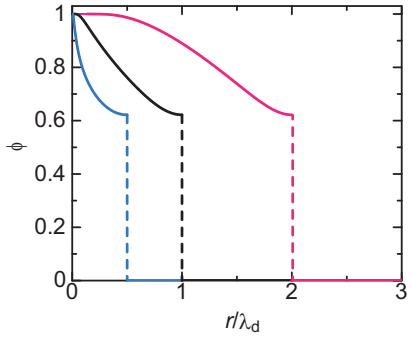

Fig. 4 The volume fraction $\phi(r,t)$ of arcRNA is shown as a function of the distance r from the DNA locus, at which arcRNA is produced, for $4\pi\lambda_p/\lambda_d = 24.8$ (magenta), 2.84 (black), and 0.337 (cyan), see eqs. (10) and (13) for the definition of λ_d and λ_p. We used $\chi = 1.0$, $\beta\varepsilon = -2.0$, and $N = 100$ for the calculations.

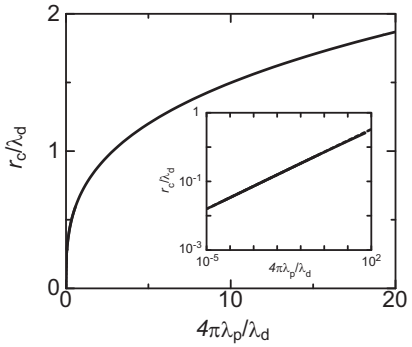

Fig. 5 The radius r_c of the condensate is shown as a function of the production rate $4\pi\lambda_p/\lambda_d$ of arcRNA, see eqs. (10) and (13) for the definition of λ_d and λ_p. We used $\chi = 1.0$, $N = 100$, $\beta\varepsilon = -2.0$ for the calculations. It is also shown as the double log plot (the solid curve in the inset) and we obtained $r_c \sim (\lambda_p/\lambda_d)^{0.33}$ by curvefitting (the broken curve in the inset).

The volume fraction of arcRNA is large at the proximity of the DNA locus and it decreases with increasing the distance r from the locus, see fig. 4. When the interaction parameter χ is larger than the critical value χ_c, the volume fraction jumps at a distance r_c from the DNA locus. This jump defines the interface between the condensate and the nucleoplasm. The chemical potential, the osmotic pressure, and the flux of arcRNA are continuous at the interface due to the local equilibrium condition. The continuity condition of the chemical potential and the osmotic pressure implies that the two values of the volume fraction at the interface correspond to the values of the volume fraction of the two coexisting phases in the equilibrium and are independent of the production rate of arcRNA.

The radius r_c of the condensate is an increasing function of the production rate k_p, see fig. 5. When the interaction parameter χ is large enough, the volume fraction of arcRNA is large in the condensate and is very small in the exterior solution. In such case, the flux of arcRNA that penetrates through the interface has an asymptotic form

$$J = \frac{k_B T}{N\zeta}\frac{\phi_{ex0}}{N v_0}\left(\frac{1}{r_c} + \frac{\sqrt{N}}{\lambda_d}\right), \tag{11}$$

and it is small when the volume fraction ϕ_{ex0} of arcRNA at the exterior solution side of the interface is small. Most arcRNA molecules produced at the DNA locus thus is degraded before they diffuse out from the condensate. This leads to an approximate relationship $k_p N v_0 \approx k_d r_c^3$. This leads to the form

$$r_c \approx \lambda_d \left(\frac{\lambda_p}{\lambda_d}\right)^{1/3}, \tag{12}$$

where we used another length scale

$$\lambda_p = \frac{1}{4\pi}\frac{N v_0 \zeta k_p}{k_B T}. \tag{13}$$

The approximate relationship, eq. (12), agrees with our numerical calculations, see the inset of fig. 5.

For the case in which the degradation of arcRNA is negligible ($k_d \to 0$), the solution of eq. (2) has the form

$$\beta \Pi_{osm}(\phi) v_0 = \frac{\lambda_p}{r}. \tag{14}$$

The radius of the condensate thus has the form

$$r_c = \frac{\lambda_p}{\beta \Pi_{osm}(\phi_{in0}) v_0}, \tag{15}$$

where ϕ_{in0} is the volume fraction of arcRNA in the condensate size of the interface. The radius r_c is proportional to the production rate of arcRNA. Eq. (15) is **valid** for

$$\frac{\lambda_p}{\lambda_d} < (\beta \Pi_{osm}(\phi_{in0}) v_0)^{3/2}, \tag{16}$$

see eqs. (12) and (15). The right side of eq. (16) is small for the case in which the interaction parameter is large.

5 Discussion

We used an extension of the Flory-Huggins theory to predict the phase separation driven by the production of arcRNA transcripts. Our theory predicts that the volume fraction of arcRNA jumps at a distance r_c from the DNA locus, at which arcRNA is produced, and it defines the interface between the condensate and the exterior solution (nucleoplasm). The two values of the volume fraction of arcRNA are determined by the continuity of the chemical potential and the osmotic pressure at the interface and the radius of the condensate depends on the production rate and the degradation rate of arcRNA. There are theories[22–24] and simulations[26–30] that treat the phase separation of chromatin and some of them highlight the non-equilibrium features of the phase separation[22–24,27,30]. However, these theories and simulations treat the time scale in which the number of chromatin is constant and thus the production of constituents is not the essence of the phase separation.

Our theory predicts that the jump of the volume fraction of arcRNA results from the fact that the values of the volume fraction in the miscibility gap are unstable. This result is similar to the case of the phase coexistence of a van der waals fluid between two heat baths of different temperature[31]. **In a fine resolution, the interface has a finite thickness and the two equilibrium values of the volume fraction of arcRNA at the interface are interpolated by a continuous profile. The profile is predicted by using the Lifshitz free energy, $\beta f_L v_0 = \frac{b^2}{24\phi}(\nabla\phi)^2$, that takes into account the conformational entropy of arcRNA by the leading order[32]. The interfacial tension of condensates is predicted by taking into account the Lifshitz free energy in an extension of our present theory. However, we neglect this free energy to highlight the roles played by the transcription dynamics in the phase separation.**

Among the parameters involved in our theory, the production rate k_p and degradation rate k_d of arcRNA may be determined by single molecule experiments. It is a better approximation to estimate N by the number of the binding site of binding proteins[11], than the length of arcRNA divided by the Kuhn length of RNA. The interaction parameter χ is routinely used in polymer physics, however, the values of the binding proteins on arcRNA are not known. The phase separation is suppressed when the number N of segments is smaller than a threshold value. The interaction parameter may be estimated by comparing our theory and the threshold value of N that is determined experimentally. The latter approach assumes that the interaction parameter does not depend on the number N of segments. The binding energy ε may be estimated by measuring the occupancy of binding proteins of an isolated arcRNA. The friction constant ζ may be estimated from the diffusion constant $k_B T/(N\zeta)$, assuming that the hydrodynamic interactions between segments are screened by the crowded environment of condensates in a nucleus.

Although our theory is relatively generic to nuclear bodies that form due to phase separation, it is ideally tested by experiments with which 1) arcRNA is randomly distributed in the condensate, 2) the radius of the condensate is much larger than the radius of gyration of arcRNA, 3) the production of arcRNA is approximately constant, not like transcription burst, 4) the intermolecular RNA-RNA interactions during transcription are negligible, and 5) the hydrodynamic interactions between segments are negligible. Paraspeckles are scaffolded by Neat1_2 arcRNA[8–11]. **Our theory does not (or only implicitly) take into account the excluded volume of binding proteins (either bound or unbound). Moreover, we have used the local equilibrium approximation to the binding of proteins to arcRNAs. The latter approximation is motivated by the fact that proteins can bind to nascent arcRNAs before the production of these transcripts is terminated[33–35]. Theoretically, the local equilibrium approximation is safely applicable to the analysis of the steady state because the local concentration of proteins does not change as a function of time (much like the situation of counterions that neutralize ionic products of electrochemical reactions[36]).** The two ends of Neat1_2 occupies the corona (shell) and the middle region of this arcRNA occupies the core[11,37,38], analogous to polymer micelles. The long-range interactions due to the tri-block nature of Neat1_2 may be treated by using Ohta-Kawasaki free energy[39,40], a density functional theory[41], or a phenomenological approach[42]. Transcriptional condensates and nucleoli are assembled through the phase separation[2,3,7,43]. Chromatin is grafted to the surface of transcriptional condensates via RNA polymerase[4,44,45] or (su-

per)enhancers[2,3,46,47], analogous to microemulsion. The chromatin at the surface may be treated as a polymer brush[47]. The nucleolar protein condensates form probably through the classical phase separation and the growth of these condensates to nucleoli at the two nucleolar organizing centers is driven by the transcription of rRNA[7]. These condensates may be treated by using an extension of our theory.

Our theory predicts that the radius r_c scales as $(Nk_p)^{1/3}$ to the production rate k_p and the number of segments of arcRNA N, see eq. (12), but the condition of the two-phase coexistence does not depend on the production rate. This result may be accessible by experiments that measure the radius of the condensate by changing the transcription rate of arcRNA and/or by decreasing the length of arcRNA by CRISPR/Cas9[11]. **Indeed, eq. (12) predicts that the number of arcRNAs in a condensate is $\frac{4\pi r_c^3}{3Nv_0} \approx \frac{k_p}{k_d}$. The time necessary for the *de novo* assembly of paraspeckles composed of ~ 100 arcRNAs takes in the order of 1 hour[11,12], whereas the life time of arcRNAS (Neat1_2) is in the order of 2 hour[11]. The production and degradation rates of arcRNAs are estimated as $k_p \sim 0.01\ s^{-1}$ and $k_d \sim 1 \times 10^{-4}\ s^{-1}$. Our theory estimates the number of arcRNAs in a condensate as ~ 100, which is the correct order of the number of arcRNAs in a paraspeckle. This implies that the degradation of arcRNAs may be a limiting factor of the size of paraspeckles. Our present model is a minimal model and can be extended by taking into account the detailed structure and dynamics of nuclear bodies. Such extension may be useful to advance our understanding of the mechanism and dynamics of the assembly of nuclear bodies, such as paraspeckles[11,48] and superenhancers[2,4] that are commonly induced by transcription of specific arcRNAs.**

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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