

**Supplementary File: Nascent ribosomal RNA
acts as surfactant that suppresses growth of
fibrillar centers in nucleolus**

S1 Elastic free energy of nascent RNA transcripts

In the main article, we use an extension of the theory of polymer brush to treat nascent pre-rRNA transcripts. The Alexander approximation assumes that the volume fraction of nascent pre-rRNA units is uniform for nascent pre-rRNA transcripts on a planer surface.^{1,2} With this approximation, the free energy per unit area of nascent pre-rRNA transcripts on a planer surface has the form

$$\frac{F_{\text{bru}}}{k_{\text{B}}T} = \frac{3}{2} \frac{\sigma h^2}{N_{\text{r}} b^2} + v \sigma N_{\text{r}} \frac{\sigma N_{\text{r}}}{h}. \quad (\text{S1})$$

This free energy is a function of the height h of the nascent pre-rRNA transcripts, see fig. 3 in the main article. The first term of eq. (S1) is the elastic free energy of nascent pre-rRNA transcripts and the second term of eq. (S1) is the free energy due to the interactions between nascent pre-rRNA units. k_{B} is the Boltzmann constant and T is the absolute temperature. N_{r} is the number of units in a nascent pre-rRNA transcript and b is the length of each unit. σ is the surface density of nascent pre-rRNA transcripts. v is the “excluded volume” that represents the magnitude of the interactions between nascent pre-rRNA transcripts divided by the thermal energy $k_{\text{B}}T$ (and has a relationship with the interaction parameter χ introduced in eq. (7) in the main article). Eq. (S1) is rewritten in the form

$$\frac{F_{\text{bru}}}{k_{\text{B}}T} = \frac{3}{2} \frac{b^4 \sigma^3 N_{\text{r}}}{\phi_{\text{r}}^2} + \frac{v}{b^3} \sigma N_{\text{r}} \phi_{\text{r}} \quad (\text{S2})$$

by using the form of the volume fraction of nascent pre-rRNA units

$$\phi_{\text{r}} = \frac{b^3 \sigma N_{\text{r}}}{h}. \quad (\text{S3})$$

Minimizing eq. (S1) with respect to h leads to

$$h = N_{\text{r}} b \left(\frac{\sigma v}{3b} \right)^{1/3}. \quad (\text{S4})$$

The volume fraction of nascent pre-rRNA units is derived as

$$\phi_r = \left(\frac{3\sigma^2 b^7}{v} \right)^{1/3} \quad (\text{S5})$$

by substituting eq. (S4) into eq. (S5) or by minimizing eq. (S2) with respect to ϕ_r . Eq. (S5) is indeed equivalent to eq. (15) in the main article (by noticing the relationship $v = b^3(2-\chi)$).

Eq. (S1) is rewritten in the following form

$$\frac{F_{\text{bru}}}{k_{\text{B}}T} = \frac{\sigma N_r}{g} \left[\frac{3}{2} \frac{\xi^2}{gb^2} + vg \frac{g}{\xi^3} \right]. \quad (\text{S6})$$

With this expression, we divide each nascent pre-rRNA into blobs of size ξ ($\equiv \sigma^{-1/2}$), which is the average distance between points, at which two neighboring nascent pre-rRNA transcripts are end-grafted to the surface. g is the number of the subchain of nascent pre-rRNA units in each blob. The first term in the square bracket in eq. (S6) is the elastic free energy of the subchain in a blob and the second term in the square bracket is the free energy due to the interactions between nascent pre-rRNA units in the blob. The prefactor N_r/g is the number of blobs in each nascent pre-rRNA transcript. Eq. (S6) returns to eq. (S2) by using the form of the local volume fraction of nascent pre-rRNA units

$$\phi_r = \frac{b^3 g}{R^3}. \quad (\text{S7})$$

Now we derive the free energy of nascent pre-rRNA transcripts end-grafted to a spherical surface at the distance r_{in} from the center, see fig. 1 in the main article. We divide each nascent pre-rRNA into blobs of size

$$\xi = \frac{r}{\sigma^{1/2} r_{\text{in}}}. \quad (\text{S8})$$

Eq. (S8) is derived by using the fact that the spherical surface at the distance r from the

center of this surface is occupied by $4\pi r_{\text{in}}^2 \sigma$ transcripts, where the area of the blob of each transcript is ξ^2 :

$$4\pi r^2 = (4\pi r_{\text{in}}^2 \sigma) \xi^2. \quad (\text{S9})$$

In the spirit of the Daoud-Cotton theory,³ eq. (S6) is extended to

$$\frac{F_{\text{brn}}}{k_{\text{B}}T} = \int_{r_{\text{in}}}^{r_{\text{ex}}} \frac{4\pi r^2 dr}{\xi^3} \left[\frac{3}{2} \frac{\xi^2}{gb^2} + vg \frac{g}{\xi^3} \right], \quad (\text{S10})$$

where $4\pi r^2 dr / \xi^3$ is the number of blobs in the spherical shell of thickness dr at the distance r from the center. Eq. (S10) is rewritten as

$$\frac{F_{\text{brn}}}{k_{\text{B}}T} = \int_{r_{\text{in}}}^{r_{\text{ex}}} \frac{4\pi r^2 dr}{b^3} \left[\frac{3}{2} \frac{b^4 \sigma^2 r_{\text{in}}^4}{r^4} \frac{1}{\phi_{\text{r}}} + \frac{v}{b^3} \phi_{\text{r}}^2 \right]. \quad (\text{S11})$$

The first term in the square bracket of eq. (S11) is the elastic free energy of the chain subsection in a blob of nascent pre-rRNA transcripts and the second term in the square bracket of eq. (S11) is the free energy due to the interactions between nascent pre-rRNA units in the blob. The first term of eq. (S11) is equal to eq. (5) in the main article.

S2 Chemical potential and osmotic pressure

The free energy density of the dense fibrillar component (DFC) has the form

$$\begin{aligned} \frac{\omega}{k_{\text{B}}T} &= \frac{3}{2} \frac{1}{\phi_{\text{r}}} \frac{\sigma^2 b^4}{r^4} r_{\text{in}}^4 - \chi (\phi_{\text{p}} + \alpha_{\text{p}} \phi_{\text{r}})^2 \\ &+ \phi_{\text{r}} (\alpha_{\text{p}} \log \alpha_{\text{p}} + (1 - \alpha_{\text{p}}) \log(1 - \alpha_{\text{p}}) - \epsilon \alpha_{\text{p}}) \\ &+ \phi_{\text{p}} \log \phi_{\text{p}} + (1 - \phi_{\text{p}} - (1 + \alpha_{\text{p}}) \phi_{\text{r}}) \log(1 - \phi_{\text{p}} - (1 + \alpha_{\text{p}}) \phi_{\text{r}}) \end{aligned} \quad (\text{S12})$$

see eqs. (3) - (8) in the main article. The free energy is the functional of the volume fraction ϕ_{r} of nascent pre-rRNA unit, the volume fraction ϕ_{p} of RNA-binding proteins (RBPs) freely

diffusing in the DFC layer, and the occupancy α_p of nascent pre-rRNA units by RBPs. $-\epsilon k_B T$ is the energy due to the binding of a RBP to a nascent pre-rRNA unit. χ is the interaction parameter, which is the magnitude of the interactions between RBPs divided by the thermal energy $k_B T$. σ is the surface density of nascent pre-rRNA transcripts end-grafted to the surface of the fibrillar center (FC) via RNA polymerase I (pol I).

With the chemical potential μ_p and osmotic pressure Π , the free energy density has the form

$$f = \omega - \mu_p(\phi_p + \alpha_p \phi_r) + \Pi b^3. \quad (\text{S13})$$

The free energy F of the system has the form

$$\frac{F}{V_m} = \frac{3}{4\pi r_{\text{in}}^3} \left[F_d + 4\pi r_{\text{in}}^2 \gamma_{\text{in}} + 4\pi r_{\text{ex}}^2 \gamma_{\text{ex}} \right], \quad (\text{S14})$$

where F_d is the free energy of a DFC layer

$$F_d = \int_{r_{\text{in}}}^{r_{\text{ex}}} 4\pi r^2 dr f. \quad (\text{S15})$$

γ_{in} and γ_{ex} are surface tension at the interface between the FC and the DFC at $r = r_{\text{in}}$ and the interface between the GC and the DFC at $r = r_{\text{ex}}$ and have the forms

$$\gamma_{\text{in}} = \gamma_p(\phi_p(r_{\text{in}}) + \alpha_p(r_{\text{in}})\phi_r(r_{\text{in}})) \quad (\text{S16})$$

$$\gamma_{\text{ex}} = \gamma_p(\phi_p(r_{\text{ex}}) + \alpha_p(r_{\text{in}})\phi_r(r_{\text{ex}})), \quad (\text{S17})$$

where γ_p is the surface tension due to the RBPs. Eq. (S15) should be minimized with the constraint

$$\int_{r_{\text{in}}}^{r_{\text{ex}}} dr 4\pi r^2 \phi_r = 4\pi r_{\text{in}}^2 \sigma N_r, \quad (\text{S18})$$

with which the number of pre-rRNA units in a DFC layer is constant.

By minimizing the free energy f with respect to ϕ_p and ϕ_r with eq. (S18), we derive the thermodynamic relationships

$$\mu_p = \frac{\partial \omega}{\partial \phi_p} \quad (\text{S19})$$

$$\Pi b^3 = \phi_r^2 \frac{\partial}{\partial \phi_r} \left(\frac{\omega}{\phi_r} \right). \quad (\text{S20})$$

By using eq. (S12), the chemical potential μ_p and the osmotic pressure Π are derived as

$$\begin{aligned} \frac{\mu_p}{k_B T} &= \log \phi_p - \log(1 - \phi_p - (1 + \alpha_p)\phi_r) \\ &\quad - 2\chi(\phi_p + \alpha_p\phi_r) \end{aligned} \quad (\text{S21})$$

$$\begin{aligned} \frac{\Pi b^3}{k_B T} &= -(1 + \alpha_p)\phi_r - \log(1 - \phi_p - (1 + \alpha_p)\phi_r) \\ &\quad - \chi(\phi_p + \alpha_p\phi_r)^2 - \frac{3(\sigma b^2)^2 r_{\text{in}}^4}{\phi_r r^4}, \end{aligned} \quad (\text{S22})$$

see also eqs. (12) and (13) in the main article.

S3 Volume fraction of nascent RNA units

The local volume fractions, ϕ_r and ϕ_p , of nascent RNA units and RBPs freely diffusing in the DFC layer are derived by using the assumption that the chemical potential μ_p of RBPs and the osmotic pressure Π are uniform in the DFC layer, see also eq. (S21) and (S22). We treat cases in which the binding energy ϵ is relatively large and the occupancy α_p of RBPs on nascent RNA units is (approximately) unity even for cases in which the volume fraction of RBPs is small. In this section, we show the derivation of the asymptotic forms of the volume fraction ϕ_r of nascent RNA units and the volume fraction ϕ_p of RBPs freely diffusing in the DFC layer.

S3.1 Small RNA volume fraction

For cases in which the volume fractions of nascent pre-rRNA units and RBPs are both small, eqs. (S21) and (S22) has asymptotic forms

$$\frac{\mu_p}{k_B T} = \log \phi_p + (\phi_p + 2\phi_r) - 2\chi(\phi_p + \phi_r) \quad (\text{S23})$$

$$\frac{\Pi b^3}{k_B T} = -\frac{3(\sigma b^2)^2 r_{\text{in}}^4}{\phi_r r^4} + \phi_p + \frac{1}{2}(\phi_p + 2\phi_r)^2 - \chi(\phi_p + \phi_r)^2, \quad (\text{S24})$$

which are derived by expanding eqs. (S21) and (S22) in the power series of ϕ_r and ϕ_p and by omitting the higher order terms. For cases in which the volume fraction ϕ_p of RBPs is very small, $\phi_p \approx 0$, eqs. (S23) and (S24) are further simplified to

$$\frac{\mu_p}{k_B T} = \log \phi_p + 2\phi_r - 2\chi\phi_r \quad (\text{S25})$$

$$\frac{3(\sigma b^2)^2 r_{\text{in}}^4}{\phi_r r^4} = 2\phi_r^2 - \chi\phi_r^2. \quad (\text{S26})$$

We assumed $\Pi b^3/(k_B T) \approx 0$ to derive eq. (S26). The volume fraction ϕ_r of nascent pre-rRNA transcripts is derived as

$$\phi_r = \left(\frac{3\sigma^2 b^4}{2 - \chi} \right)^{1/3} \left(\frac{r_{\text{in}}}{r} \right)^{4/3}. \quad (\text{S27})$$

by solving eq. (S26) with respect to ϕ_r . Eq. (S27) reduces to eq. (15) in the main article for $(r_{\text{ex}} - r_{\text{in}}) \ll r_{\text{in}}$. The volume fraction of RBPs is derived by substituting eq. (S27) into eq. (S25). In this asymptotic limit, the volume fraction of solvent molecules is dominant in the dense fibrillar component (DFC) layer (which is defined by the region, where nascent pre-rRNA transcripts are localized).

For cases in which the volume fraction of RBPs is approximately unity, $\phi_p \approx 1$, eqs.

(S21) and (S22) have asymptotic forms

$$\frac{\mu_p}{k_B T} = -\log(1 - \phi_p - 2\phi_r) - 2\chi \quad (\text{S28})$$

$$\frac{\Pi b^3}{k_B T} = -\log(1 - \phi_p - 2\phi_r) - \chi - \frac{3(\sigma b^2)^2 r_{\text{in}}^4}{\phi_r r^4}. \quad (\text{S29})$$

By eliminating the factor $-\log(1 - \phi_p - (1 + \alpha_p)\phi_r)$ from eq. (S29) by using eq. (S28), the osmotic pressure has the form

$$\frac{\Pi b^3}{k_B T} = \frac{\mu_p}{k_B T} + \chi - \frac{3(\sigma b^2)^2 r_{\text{in}}^4}{\phi_r r^4}. \quad (\text{S30})$$

The volume fraction of nascent RNA units is derived as

$$\phi_r = \frac{3\sigma^2 b^4}{\chi + \mu_p/(k_B T)} \frac{r_{\text{in}}^4}{r^4} \quad (\text{S31})$$

by solving eq. (S30) with respect to ϕ_r and by using the assumption $\Pi b^3/(k_B T) \approx 0$. Eq. (S31) is equivalent to eq. (22) in the main article and is reduced to eq. (16) in the main article for $(r_{\text{ex}} - r_{\text{in}}) \ll r_{\text{in}}$. The volume fraction of RBPs is derived as

$$\phi_p = 1 - e^{-2\chi - \mu_p/(k_B T)} - \frac{6\sigma^2 b^4}{\chi + \mu_p/(k_B T)} \frac{r_{\text{in}}^4}{r^4}, \quad (\text{S32})$$

by substituting eq. (S31) into eq. eq. (S28).

S3.2 Large RNA volume fraction

For cases in which the volume fraction of nascent RNA units is approximately 1/2, it is convenient to represent eq. (S21) and (S22) in terms of the volume fractions, ϕ_p and ϕ_s , of RBPs and solvent molecules with

$$\phi_r = \frac{1}{2}(1 - \phi_p - \phi_s). \quad (\text{S33})$$

For small values of the volume fractions, ϕ_p and ϕ_s , eqs. (S21) and (S22) have asymptotic forms

$$\frac{\mu_p}{k_B T} = \log \phi_p - \log \phi_s - \chi(1 + \phi_p - \phi_s) \quad (\text{S34})$$

$$\frac{\Pi b^3}{k_B T} = -\log \phi_s - \frac{6(\sigma b^2)^2 r_{\text{in}}^4}{r^4} - 1 - \frac{\chi}{4}, \quad (\text{S35})$$

which are derived by substituting eq. (S33) into eqs. (S21) and (S22) and expanding these equations with respect to ϕ_p and ϕ_s . The volume fractions, ϕ_p and ϕ_s , are derived as

$$\phi_p = \phi_s e^{\frac{\mu_p}{k_B T} + \chi} \quad (\text{S36})$$

$$\phi_s = e^{-\frac{\chi}{4} - 1 - \frac{6(\sigma b^2)^2 r_{\text{in}}^4}{r^4}} \quad (\text{S37})$$

by using eqs. (S34) and (S35) and the assumption $\Pi b^3/(k_B T) \approx 0$. The volume fraction of nascent RNA units is derived as

$$\phi_r = \frac{1}{2} \left(1 - \left(1 + e^{\frac{\mu_p}{k_B T} + \chi} \right) e^{-\frac{\chi}{4} - 1 - \frac{6(\sigma b^2)^2 r_{\text{in}}^4}{r^4}} \right) \quad (\text{S38})$$

by substituting eqs. (S36) and (S37) into eq. (S33). Eq. (S38) is equivalent to eq. (23) in the main article.

S4 Approximate form of radius of fibrillar centers

S4.1 Mean field theory

For cases in which the volume fraction of nascent pre-rRNA is approximately 1/2, the free energy has an approximate form

$$\frac{f}{k_B T} \simeq \frac{3\sigma^2 b^4}{r^4} r_{\text{in}}^4 - \frac{1}{2}\epsilon - \frac{1}{4}\chi - \frac{1}{2} \frac{\mu_p}{k_B T}, \quad (\text{S39})$$

where it is derived by substituting eq. (S33) into eq. (S13), by expanding it with respect to ϕ_p and ϕ_s , and by omitting the higher order term. Eq. (S39) implies that the elastic free energy dominates other free energy contributions in this asymptotic limit. By using eq. (S39), the free energy of the DFC layer has the form

$$\frac{F_d b^3}{3k_B T V_m} \simeq 3\sigma^2 b^4 \left(1 - \frac{r_{\text{in}}}{r_{\text{ex}}}\right), \quad (\text{S40})$$

see eq. (S15).

The exterior radius r_{ex} is derived as

$$\frac{r_{\text{ex}}}{r_{\text{in}}} = \left(1 + \frac{6N_r \sigma b^3}{r_{\text{in}}}\right)^{1/3} \quad (\text{S41})$$

by substituting eq. (S33) into eq. (S18), by expanding in a power series of ϕ_p and ϕ_s , and by omitting the higher order terms.

With eq. (S41), the free energy has the form

$$\begin{aligned} \frac{F b^3}{3k_B T V_m} &= 3(\sigma b^2)^2 \left(1 - \left(1 + \frac{6N_r \sigma b^3}{r_{\text{in}}}\right)^{-1/3}\right) \\ &\quad + \frac{1}{2} \frac{\gamma_p b^2}{k_B T} \frac{b}{r_{\text{in}}} \left(1 + \left(1 + \frac{6N_r \sigma b^3}{r_{\text{in}}}\right)^{2/3}\right) \end{aligned} \quad (\text{S42})$$

The surface density σ of nascent pre-rRNA is proportional to the radius r_{in} , see eq. (2) in the main article. By minimizing eq. (S42) with respect to the radius r_{in} , the surface density σ is derived as

$$\sigma b^2 = \left(\frac{\gamma_p b^2}{12N_r k_B T}\right)^{1/3} \left[\frac{\frac{N_r \sigma b^3}{r_{\text{in}}} \left(1 + \left(1 + \frac{6N_r \sigma b^3}{r_{\text{in}}}\right)^{2/3}\right)}{1 - \left(1 + \frac{6N_r \sigma b^3}{r_{\text{in}}}\right)^{-1/3}}\right]^{1/3}. \quad (\text{S43})$$

For $N_r \sigma b^3 / r_{\text{in}} < 1$, eq. (S43) has an approximate form

$$\sigma b^2 = \left(\frac{\gamma_p b^2}{12 N_r k_B T} \right)^{1/3} \left(1 + \frac{6 N_r \sigma b^3}{r_{\text{in}}} \right)^{1/3}, \quad (\text{S44})$$

which is derived by expanding the square bracket in eq. (S43) in a power series of $N_r \sigma b^3 / r_{\text{in}}$ and by omitting the higher order terms. Eq. (S44) is equivalent to eq. (25) in the main article.

S4.2 Scaling theory

The mean field theory does not take into account the correlation due to the excluded volume interactions between nascent pre-rRNA units in the elastic free energy and the correlation due to the connectivity of nascent pre-rRNA units in the interaction free energy.⁴ The free energy of nascent pre-rRNA is better estimated by dividing nascent pre-rRNA transcripts into blobs and by assuming that each blob has the free energy of the order of $k_B T$ (see sec. S1 for the division of nascent pre-rRNA transcripts into blobs). With this theory, the free energy of the system is derived as

$$F_d = k_B T \tilde{c}_0 \int_{r_{\text{in}}}^{r_{\text{ex}}} dr \frac{4\pi r^2}{\xi^3}. \quad (\text{S45})$$

\tilde{c}_0 is the numerical factor of order unity and is not determined by the scaling theory. Eq. (S45) is rewritten as

$$\frac{F_d b^3}{3 k_B T V_m} = \tilde{c}_0 (\sigma b^2)^{3/2} \log \frac{r_{\text{ex}}}{r_{\text{in}}}. \quad (\text{S46})$$

The free energy of the system has the form

$$\frac{F b^3}{3 k_B T V_m} = \frac{1}{3} \tilde{c}_0 (\sigma b^2)^{3/2} \log \left(1 + \frac{6 N_r \sigma b^3}{r_{\text{in}}} \right)$$

$$+ + \frac{1}{2} \frac{\gamma_p b^2}{k_B T} \frac{b}{r_{\text{in}}} \left(1 + \left(1 + \frac{6N_r \sigma b^3}{r_{\text{in}}} \right)^{2/3} \right). \quad (\text{S47})$$

By minimizing the free energy with respect to the radius r_{in} , the surface density σ is derived as

$$\sigma b^2 = c_0 \left(\frac{\gamma_p b^2}{N_r k_B T} \right)^{2/5} \left[\frac{\frac{N_r \sigma b^3}{r_{\text{in}}} \left(1 + \left(1 + \frac{6N_r \sigma b^3}{r_{\text{in}}} \right)^{2/3} \right)}{\log \left(1 + \frac{6N_r \sigma b^3}{r_{\text{in}}} \right)} \right]^{2/5}, \quad (\text{S48})$$

where c_0 is the numerical factor of order unity, $c_0 = \tilde{c}_0^{-2/5}$. For $N_r \sigma b^3 / r_{\text{in}} < 1$, eq. (S48) has an approximate form

$$\sigma b^2 = c_0 \left(\frac{\gamma_p b^2}{N_r k_B T} \right)^{2/5} \left(1 + \frac{5N_r \sigma b^3}{r_{\text{in}}} \right)^{2/5}, \quad (\text{S49})$$

which is derived by expanding the inside of the square bracket in eq. (S48) in a power series of $N_r \sigma b^3 / r_{\text{in}}$ and by omitting the higher order terms. Eq. (S49) is equivalent to eq. (26) in the main article.

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