

Supplementary information

Movies

Movie 1-8. 3D reconstruction of the ER network by EM tomography for the following genotypes: *atl*² (movie 1-2), *Rtnl1*¹/*atl*² (movie 3-4), control (movie 5-6), *Rtnl1*¹ (movie 7-8). Scale bar 200nm.

Movie 9, 10 Examples of the scission of individual ER branches in COS-7 cells overexpressing Rtnl1. The movies were taken at 100 frames/s. The frame sequence corresponding to Movie 9 is shown in Fig. 3c.

Movie 11. Fission of the nanotube pulled from Rtnl1-containing membrane reservoir. The movie was recorded at 30 frames/s. The frame sequence corresponding to the movie is shown in Fig. 4c.

Theoretical analysis of dynamic membrane constriction by Rtnl1

Outline of the model. We consider a cylindrical nanotube pulled from a planer reservoir membrane at a constant velocity v_t . We assume that at rest ($V_t=0$) Rtnl1 is symmetrically distributed between the monolayer of the reservoir membrane while it resides preferably in the outer (positively curved) monolayer of the tube. The curvature preference is determined by intrinsic membrane curvature (J_s) produced by Rtnl1 and/or its oligomers^{1,2}. Rtnl1 insertion/polymerization in the monolayers augments surface viscosity of the monolayers proportionally to Rtnl1 concentration. We further assume that during the nanotube elongation Rtnl1 migrates together with lipids to minimize friction losses. The migration is coupled to curvature-driven sorting of Rtnl1. Consider the outer monolayer of the nanotube. As the Rtnl1 concentration there is higher than in the planar monolayer of the reservoir membrane, the nanotube elongation creates a diffusion flux in the planar monolayer towards the nanotube. This coupling results in Rtnl1 depletion in the planar monolayer near the nanotube, proportional to V_t . The Rtnl1 depletion diminishes the integral viscose resistance to pulling; for sufficiently strong curvature sorting, the corresponding increase of Rtnl1 concentration in the reservoir monolayer coupled to the inner monolayer of the nanotube can be neglected. Hence, the Rtnl1 depletion provides a plausible explanation for the shear-thinning effect observed experimentally (Fig. 4e).

Protein transport equation.

We considered a nanotube pulled from the reservoir membrane at a constant speed V_t . Rtnl1 flux I in the tube is:

$$(1) I = 2\pi R_t v_t c_t$$

where c_t is the Rtnl1 concentration in the nanotube and R_t is the nanotube radius (we assume that the nanotube preserves cylindrical shape during elongation)

The protein flux in the reservoir membrane is defined by the gradient of Rtnl1 concentration $c(r)$:

$$(2) 2\pi r D \frac{dc}{dr} + 2\pi r v c = I$$

From continuity of the lipid bilayer

$$(3) v(r)r = v_r R_t$$

we obtain:

$$(4) \frac{dc}{dr} + \frac{v_t R_r (c - c_t)}{D r} = 0$$

Assuming $\frac{v_t R_r}{D} \ll 1$, the solution of (4) is:

$$(5) c(r) = c_0 + (c_t - c_0) \left[1 - \left(\frac{R}{r} \right)^{\left(\frac{v_t R_r}{D} \right)} \right] \sim c_0 - (c_t - c_0) \frac{v_t R_t}{D} \ln \frac{R}{r}$$

where R is a characteristic reservoir length (e.g. the diameter of the support bead)

Sorting in elongating tubes

In a static nanotube, the sorting is defined by the stationary concentration difference between the nanotube (c_t) and the planar part ($c(R_t)$)

$$(6) c_t = c(R_t) e^{\frac{\Delta w}{k_B T}}$$

where $\Delta w \approx \frac{K J_s a}{2 R_t}$ is the energy gain (per molecule) in Rtnl1 transfer from a planar to curved lipid monolayer. From 5 and 6 we obtains:

$$(7) c_t = c_p^0 \exp\left(\frac{R_j}{R_t}\right) \frac{\frac{v_t}{v_D} + 1}{\exp\left(\frac{R_j}{R_t}\right) \frac{v_t}{v_D} + 1}$$

and

$$(7a) c(R_t) = c_p^0 \frac{\frac{v_t}{v_D} + 1}{\exp\left(\frac{R_j}{R_t}\right) \frac{v_t}{v_D} + 1}$$

where $D_1 = \frac{D}{\ln \frac{R}{R_t}}$ is a renormalized diffusion coefficient (further assumed constant), $v_D = \frac{D_1}{R_t}$ and

$R_j = \frac{k}{2 k_B T} J_s a$ is a characteristic length associated with sorting. Note that at large V_t c_t approaches c_p

so that the nanotube pulling inhibits sorting.

Axial force and constriction

In cylindrical approximation, the elastic energy of the nanotube W can be written as:

$$(9) W = 2\pi R_t L \left(\frac{k}{2 R_t^2} + \sigma \right)$$

where σ is the lateral membrane tension in the planar reservoir far from the nanotube. We assume that the reservoir membrane is symmetric (monolayers are equivalent) and R_t quickly follows changes in f . Then from 9 it follows that³:

$$(10) f = \frac{2\pi k}{R_t}$$

Note that (10) can be rewritten as $k = \frac{R_t k}{2\pi}$ to obtain effective bending rigidity from force/radius measurements (Fig. 4g). For pure lipid nanotubes it yields $k = 0.9 \cdot 10^{-19} \text{J}$, consistent with published data⁴.

If Rtnl1 distribution between the monolayers of the reservoir membrane is asymmetric, (10) can be modified as:

$$(10a) f = 2\pi k \left(\frac{1}{R_t} - J_s \right)$$

Importantly, in both cases the constriction-force relation remains the same:

$$(11) \Delta r = 2\pi k \Delta \left(\frac{1}{f} \right),$$

Eq. 11 was used to recalculate membrane constriction from

Viscous friction and pulling force

The work produced by the pulling force $f v_t$ goes to frictional dissipation Q and changes of W .

$$(12) \frac{dW}{dt} + Q = f v_t$$

Dissipation in the planar part is calculated as⁵:

$$(11) Q = 4 \int_{R_t}^R \eta(r) \left(\frac{v_t R_t}{r^2} \right)^2 2\pi r dr$$

Dissipation in the nanotube can be neglected for sufficiently long tubes (Appendix a below). In the leading order, the surface viscosity depends linearly on Rtnl1 concentration:

$$(12) \eta(r) = \eta_l(1 - ac) + \eta_p ac \approx \eta_l + \eta_p ac$$

Hence:

$$(16) Q = m_l v_t^2 + m_p (v_t) v_t^2$$

where m_l is the ‘‘lipid’’ friction $m_l = 4\pi\eta_l$ and m_p is the protein one:

$$(17) m_p = 4\pi\eta_p ac_p^0 \frac{\frac{v_t+1}{v_D}}{\exp\left(\frac{R_j}{R_t}\right) \frac{v_t+1}{v_D}}$$

Collecting the above, we obtain:

$$(18) f_0^2 = f \left(f - 2 \left(m_l + m_p(v_t) \right) v_t \right)$$

where $f_0^2 = 8\pi^2 K \sigma$ characterizes the reservoir lateral tension.

In pure lipid nanotube at low speeds $f_0 \sim f$ so that

$$(19) (f - f_0) = 4\pi\eta_l v_t$$

From (19) the viscosity η_l is estimated as $2 \cdot 10^{-6} \text{g/cm}^{3,5}$.

In Rtnl1-containing tubes f at the point of fission was always much higher than f_0 . In this case, (18) can be simplified so that Rtnl1-specific contribution to viscous resistance becomes:

$$(20) f = 2m_p(v_t, R_t)v_t$$

We further note that in our experiments $\exp\left(\frac{R_j}{R_t}\right) \frac{v_t}{v_D} \gg 1$ so that, upon subtraction of the pure lipid contribution, (17) and (20) can be combined as:

$$(21) f_{Rtnl1} \exp\left(\frac{f_{Rtnl1}}{f_s}\right) = 4\pi\eta_p a c_p^0 (v_t + v_D)$$

where $f_s = \frac{4\pi k_B T}{J_s a}$ is the effective “sorting” force. Equation (20) was used to fit the Rtnl1-specific contribution to friction resistance (Fig. 4e, insert). At large V_t , it follows from Eq. 21 that $f_{Rtnl1} \sim \ln(V_t)$. Eq. (21) was used to fit $f_{Rtnl1}(V_t)$ dependence (Fig. 4e, insert) and obtain the effective viscosity due to Rtnl1 $\eta_p a c_p^0$.

References

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