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Modeling of snRNP Motion in the Nucleoplasm

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Aim

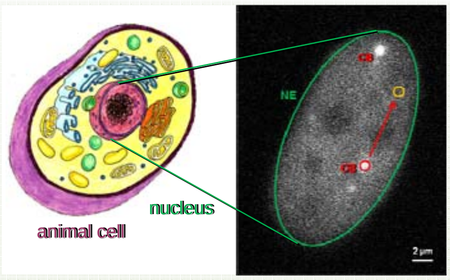
Eukaryotic cells are organized into membrane delimited organelles. Their genetic information - DNA - is stored in the nucleus. The gene expression, in which inheritable information is transformed into a functional gene product, involves processes of transcription and translation. Transcription from DNA to RNA proceeds inside the cell nucleus and translation to proteins is taking place in the cytoplasm.

Small nuclear ribonucleoproteins (snRNPs) are factors involved in pre-mRNA splicing, a two-step reaction that is a part of post-transcriptional RNA modifications. snRNPs were shown to be assembled in nuclear domains called **Cajal bodies (CBs)**.

Currently no model that would describe dynamics of the snRNPs in vivo is available. A lack of metabolic energy necessary for an active movement of intra-nuclear proteins and RNA suggests that these molecules undergo a passive diffusive movement in the nucleus. Considering this knowledge, we have tested whether the simplest model based on **free diffusion of snRNPs** could describe movement of the particles in the nucleus. Analysis of fluorescently labeled snRNP complexes located in CBs in the nucleoplasm of 50 different HeLa cell nuclei revealed that spreading of the complex into the nucleoplasm has a diffusion character, however, free diffusion model with spatially invariant diffusion coefficient could not fully explain the snRNP dynamics.

In order to eliminate a possible variability of environment in different cell nuclei we performed similar measurements and analyses on a single cell nucleus.

Experiment



HeLa cells were transfected with fluorescently labeled *SmB* protein that is taking part in the snRNP complex formation. *SmB* was tagged with photoactivatable green fluorescent protein (*PA-GFP)* that can be converted from non-fluorescent to fluorescent form by ~400 nm laser light illumination.

Diffusion of the snRNP complexes inside the nucleoplasm delimited by the nuclear envelope (**NE**, green) was visualized by photoactivation of the *SmB:PA-GFP* complex within the circular area (red circle) corresponding to one of the Cajal bodies (**CB**). Spreading of the photoactivated complexes (red arrow) was measured in the nucleoplasmic area (yellow circle).

Numerical model

Motion of snRNPs was described by a diffusion equation with constant diffusion coefficient *D*:

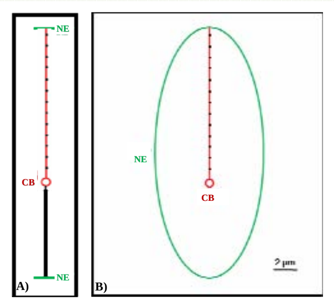
$$\frac{\partial c(x,t)}{\partial t} - D\frac{\partial^2 c(x,t)}{\partial x^2} = f \quad \text{with fundamental solution} \quad F(x-x_{CB},t) = \frac{1}{2\sqrt{\pi D t}}\, e^{-\frac{(x-x_{CB})^2}{4Dt}} \quad \text{for} \quad c(\vec{x},0) = \delta(x-x_{CB}).$$

Numerical solutions were computed in Diffusion Application Mode (Comsol). Initinal conditions $c(\vec{x},0)=c_0$ for $\vec{x} = CB$ and $c(\vec{x},0)=0$ elsewhere simulated the photoactivation in the Cajal body only. Neumann boundary conditions

$\vec{n}.\vec{N} = 0$, $\vec{N} = -D\vec{\nabla}c$ were used to define impermeability of the nuclear membrane for the diffusing particles.

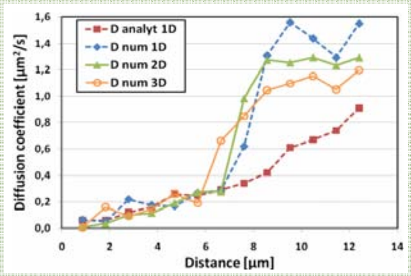
Solutions were transferred to Matlab and used for the non-linear least squares fitting of the measured fluorescence intensity evolutions.

Results - Effect of the dimensionality



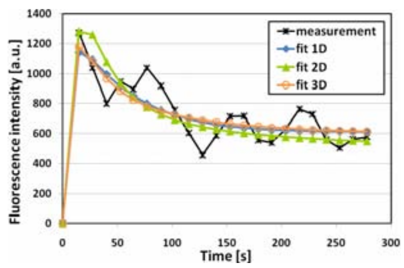
Model used for numerical 1D and 2D fitting. **A)** In 1D the cell nucleus was represented by a line segment of 21 µm length. Center of the CB (a line segment of 0.5 µm length) was located inside the nucleus, 8.2 µm from the lower NE end. The measurement points were located along the red line with 0.96 µm distance from each other. **B)** In the 2D model, the nucleus was represented by the ellipse (semi-axes of 10.5 µm and 5 µm) and the CB was modeled as a circle (with 0.25 µm radius) located on its major axis at 8.2 µm distance from the lower nuclear pole. The measurement points were located along the major axis with 0.96 µm distance from each other.

The 3D model was created from the 2D one by formation of an ellipsoid with the third semi-axis of 2.5 µm. Positions of the CB and the measurements points remained the same as for 2D model.



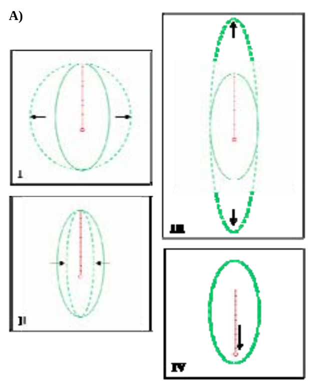
Evaluation of the diffusion coefficient in different distances from the photoactivated CB. Comparison of analytical fit in 1D with numerical fits in 1D, 2D and 3D.

For all the numerical models the results are qualitatively similar. For longer diffusion distances they all differ from the analytical 1D fit: instead of the continuous growth, the numerical solutions exhibit a sigmoidal shape of the *D(x)* curve.



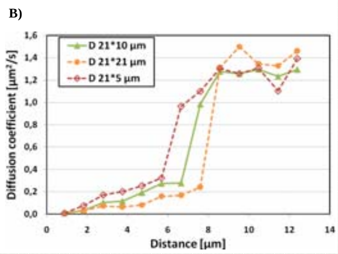
Example of experimental data measured in the distance of 2.8 µm from the photoactivated Cajal body (black crosses) and numerical fits in 1D, 2D and 3D.

Results - Effect of the geometry changes

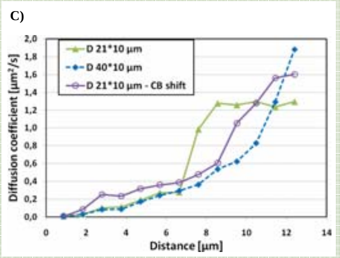


Scheme of the geometry changes in the 2D model. Elongation of the minor axis from 10 µm to 21 µm (I) and shortening of the axis from 10 µm to 5 µm (II). Elongation of the major axis from 21 µm to 40 µm (III) and change of the CB position within the nucleus (IV). The red line indicates the analysis area.

Results reveal strong dependency of the calculated diffusion coefficient on the position of the CB and the measurement points within the nucleus.



Effect of the minor axis change (configurations I and II).



Effect of the major axis elongation (III) and the CB position change (IV).

Conclusions

- **The apparent diffusion coefficient depends on the distance from the photoactivated Cajal body, found in 1D, 2D and 3D model**
  Consequence: Free diffusion does not explain the snRNP motion
- **The apparent diffusion coefficient depends on the specific model geometry**
  Consequence: Nuclear geometry is crucial for accurate modeling, each nucleus has to be modeled and analyzed separately

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