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Supporting Material

Balbiani Ring mRNPs Diffuse through and Bind to Clusters of Large Intranuclear Molecular Structures

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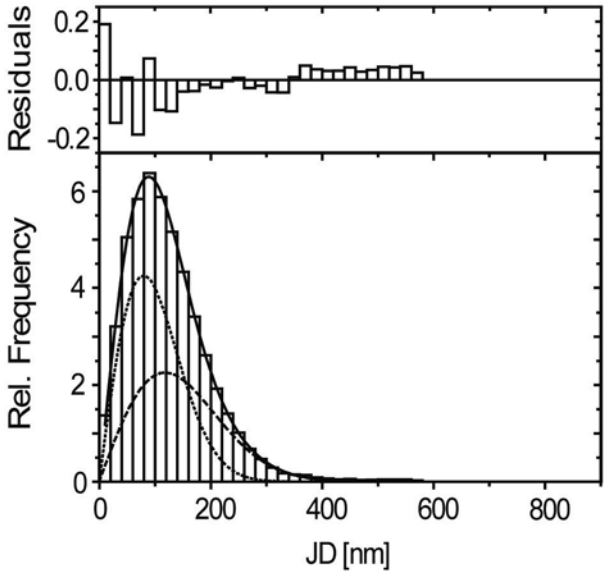
Online Supplemental Material

Supplemental Results and Discussion

Single molecule microscopy of molecular Beacon-labeled BR mRNPs

Molecular beacons (MBs) are single-stranded oligonucleotides forming a stem-and-loop structure. The loop contains an oligonucleotide segment that is complementary to a target sequence, and the stem is formed by the annealing of complementary bases that are located on both sides of the central probe segment. A fluorophore and a fluorescence quencher are covalently linked to the ends of the stem arms. Hybridization to the target sequence disrupts the stem and abolishes the quenching effect, and the probe starts to fluoresce.[1] Hence, MBs reduce the signal of unbound oligonucleotides.[2] Therefore we used 2'-O-methyl-RNA molecular beacons containing a loop sequence complementary to the repetitive sequence of the BR2.1 mRNA (designated as 2'-O-methyl-MB-RNA) in order to restrict observations to specifically bound probes. Upon microinjection of specific 2'-O-methyl-MB-RNA the BR2.1 transcription site became immediately and clearly visible in the injected nuclei (data not shown), whereas upon microinjection of unrelated MBs no BR transcription sites were revealed, indicating, the specific MBs stained BR2.1 mRNPs as expected.

BR mRNP tracking experiments, data evaluation, and trajectory analysis were performed as described above. 4 nuclei from 3 different salivary glands were analysed. OSM movie 4 shows a typical data set. Again, two diffusion components were observed with $D_{1,MB}$ = 0.3 ± 0.03 µm²/s (56 ± 12%) and $D_{2,MB}$ = 0.7 ± 0.1 µm²/s (44 ± 11%) (OSM Fig. 1). A third component was not detected. This corroborated the notion that $D_{3,SPT}$ as determined above was due to unspecifically bound probe oligonucleotides, since exactly such signals should vanish when using molecular beacons for labelling. Besides the missing D_3 the results obtained from MB oligonucleotides were in excellent agreement with those from the non-MB oligonucleotides. Again, trajectories of mRNPs labelled with MBs also frequently showed a discontinuous mobility (data not shown). With unrelated control MBs mobile particles were extremely rarely observed. Also, in the case of a particle observation the particle fluorescence intensity was much dimmer compared to the specific MBs and signal smearing indicated fast particle movement (data not shown). The signals were too weak for tracking.



OSM Figure 1: Jump distance histogram for molecular beacons

2'-O-methyl-RNA-molecular beacons (MBs) were microinjected into salivary gland cell nuclei and analyzed with single particle tracking: $D_{1,MB}$ = 0.3 ± 0.03 µm²/s with a weight of 56 ± 12% (dots) and $D_{2,MB}$ = 0.7 ± 0.1 µm²/s with a weight of 44 ± 11% (dashed). The residuals above the jump distance histograms correspond to the overall fit (solid line) due to 2 or 3 mobility components. JD, jump distance. Errors are standard errors of the mean.

Calculation of mean square displacement

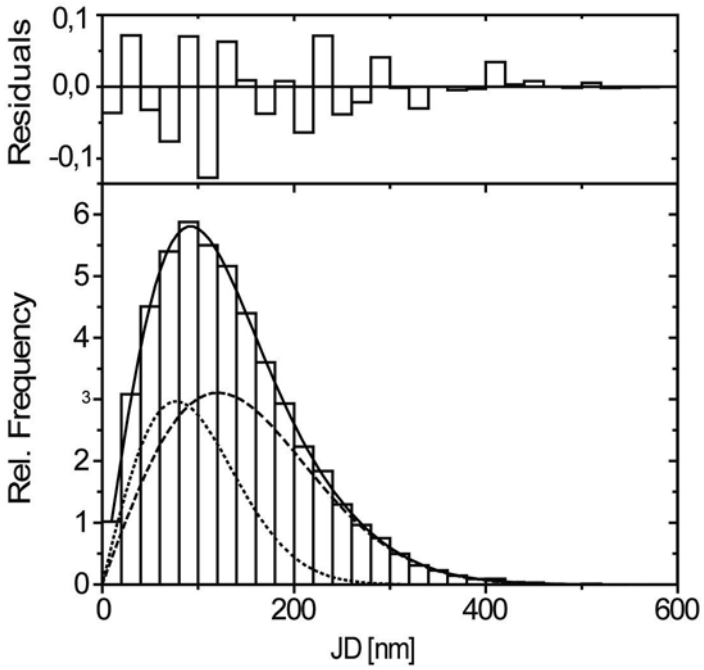
Special care was taken to avoid an overweighting of long trajectories. An example shall explain why this is important. We suppose the following szenario. 100 different trajectories are obtained in an experiment, and 99% of all trajectories show a fast mobility and are therefore detected for 11 frames each before leaving the focal plane. These trajectories move by Brownian motion with a D=5µm²/s. The remaining trajectory, however, is immobilized due to binding and is present along the entire movie length of 1000 frames. The classical way to calculate the MSD for frame m is to square all jumps of length m of the different trajectories and to calculate their mean. For the example above the following would happen, if the 10^{th} MSD is calculated: the 99 fast trajectories with a total length of 11 would each contribute *once* to the 10^{th} MSD providing 99 values. However, the single immobile trajectory would contribute 989 values to the 10^{th} MSD. Therefore the single immobile trajectory would completely dominate especially late time points.

Furthermore, it was previously shown that fast trajectories leave the focal plane rapidly[3], and later MSD points are more and more dominated by slower particles producing a non-linear MSD-time dependence in case the trajectories are not equally weighted as described above.

Binding events can lead to two mobility components found in jump distance distributions

Matlab (The MathWorks, Natick, MA, USA) was used to write a simple Monte Carlo simulation. Particles were simulated, which performed one jump according to their diffusion coefficient in the time intervall *p*, with *p* = 100µs. The corresponding jump distance was drawn from a normal distribution with a standard deviation of *sqrt(4*D*p)*. To account for binding events a particle had a chance of 0.410 to become immobilized (D = 0.001 µm^2/s) before its jump, or vice versa a bound particle had a chance of 0.095 to become mobile. These values were found to describe the experimental jump distance histogram best. The free diffusion coefficient was set to 3 µm²/s according to the diffusion of a 50 nm particle in a 3 – 5 cP solution. Our data acquisition time was 100 Hz corresponding to 10 ms integration time. Therefore every 100th simulated trajectory point was used to plot simulated trajectory. Thus frequent binding and unbinding steps occurred within an acquisition time of 10 ms. Without binding the expected single diffusion coefficient was obtained in the jump distance analysis of the simulated trajectories (data not shown). Taking binding into account with the on- and off-probabilities noted above the jump distance analysis revealed two components (OSM Fig. 2). The obtained diffusion coefficients were $D_{1,Sim,Bind}$ = 0.30 ± 0.02 µm²/s (38 ± 5%) and $D_{2,Sim,Bind}$ = 0.73 ± 0.03 µm²/s (62 ± 5%). Note that these values match those obtained experimentally for the BR2.1 mRNPs.

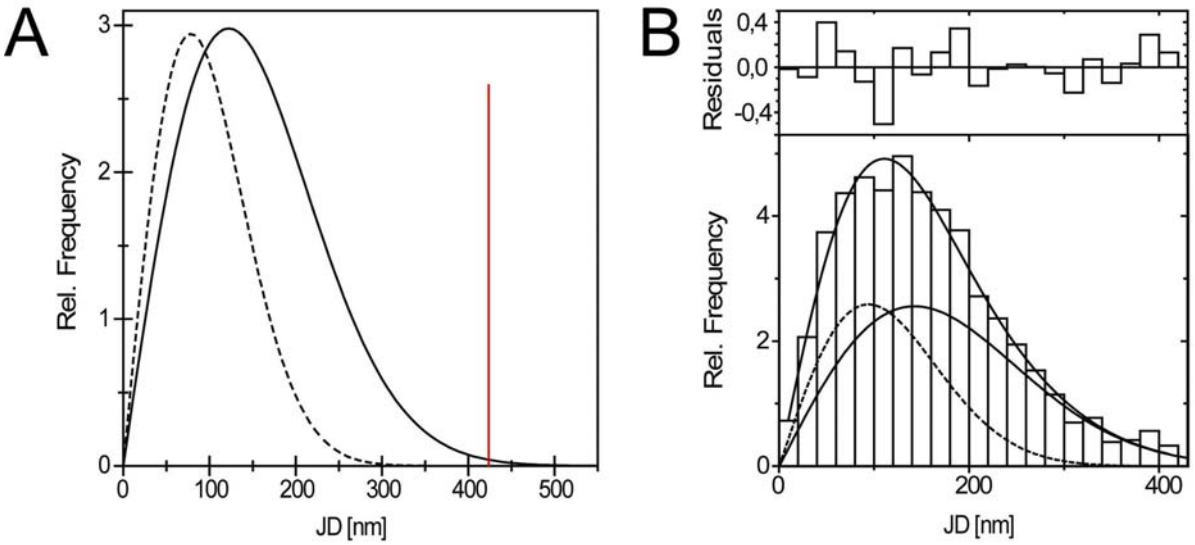
We conclude that binding processes may lead to two components in jump distance histograms. It should be noted, that these two diffusion components cannot be separated from each other, although they both occur within a single trajectory.



OSM Figure 2: Two jump distance mobility components can occur due to binding events

BR mRNP trajectories show a discontinuous movement

As described previously we were able to detect several diffusion coefficients within a single trajectory. [3] As discussed below the mobility components of 0.73 µm²/s and 0.3 µm²/s were detectable within single trajectories. OSM Fig. 3A shows the two main mobility components obtained from the jump distance analysis. Jumps of 425 nm or greater between successive frames are extremely rare for putative slow particles with a single diffusion coefficient of 0.3µm²/s when the acquisition time is ~10ms. Thus we selected only BR2.1 mRNP trajectories, which performed at least one jump of 425nm or greater. Thereby only trajectories with a high mobility were selected. However, as shown in OSM Fig. 3B, the trajectories selected required again two diffusion coefficients to fit their jump distance histogram appropriately (40%, D_1=0.43 µm²/s; 60%, D_2=1 µm²/s). In case two different particle populations with a small D of 0.3µm²/s and a high D of 0.73µm²/s are simulated with only one diffusion coefficient per trajectory, this trajectory filter only yields fast trajectories (data not shown). We conclude that the two main mobility components of 0.3 and 0.73µm²/s are present in single trajecotries.



OSM Figure 3: Single BR trajectories contain two mobility components

OSM References

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2. Bonnet G, Tyagi S, Libchaber A, Kramer FR. Thermodynamic basis of the enhanced specificity of structured DNA probes. Proceedings of the National Academy of Sciences of the United States of America 1999; 96:6171-6.

3. Siebrasse JP, Veith R, Dobay A, Leonhardt H, Daneholt B, Kubitscheck U. Discontinuous movement of mRNP particles in nucleoplasmic regions devoid of chromatin. Proceedings of the National Academy of Sciences of the United States of America 2008; 105:20291-6.

OSM Movies

OSM Movie 1: BR2.1 mRNPs mobility in live salivary gland cells

Specific oligonucleotides were microinjected into salivary gland cell nuclei to stain endogenous BR2.1 mRNPs. Data acquisition rate was 100 Hz. The movie is displayed at 20 Hz. It was slightly contrast enhanced, smoothed and shortened. The dimensions of one frame are (24.4 * 12.2) µm². A corresponding brightfield image was shown (nuclear membrane, white; Nuc Nucleus) with the vast amount of chromatin free nucleoplasm.

OSM Movie 2: Tracer mobility in live salivary gland cells

Fluorescently labeled 500kDa dextrans were microinjected into salivary gland cell nuclei. The acquisition time was 100Hz. A typical movie is displayed with 20Hz. It was contrast enhanced, smoothed and cut. Compared to the oligonucleotide stained BR2.1 mRNPs dextrans move faster. The dimensions of one frame are (24.4 * 12.2) µm². A corresponding brightfield image is shown (Nuc, Nucleus; PT Polytene Chromosomes). Dextrans do not enter the dense chromosome territories.

OSM Movie 3: Tracer mobility in live mammalian cells

Fluorescently labeled 500kDa dextrans were microinjected into mammalian H2B-GFP cell nuclei and imaged. Movie acquisition rate was 200Hz. A typical movie is shown on the right, contrast enhanced and smoothed. On the left the movie was extensively filtered by applying a Mexican hat filter followed by a background subtraction and contrast enhancement (red channel). In the background (green channel) an image of the cell is shown, which was recorded in the GFP channel before movie acquisition. Display rate, 20Hz. Dimensions, (24.4 * 12.2) µm².

OSM Movie 4: BR2.1 mRNPs mobility in live salivary gland cells

Specific molecular beacons were microinjected into salivary gland cell nuclei and imaged with 100 Hz. A typical movie is displayed with 20Hz. It was slightly contrast enhanced and shortened. Image dimensions, (24.4 * 12.2) µm². The corresponding brightfield image was shown (nuclear membrane, white; Nuc Nucleus; Cyt Cytosol; PT Polytene Chromosome). MB-labeled BR2.1 mRNPs did not enter the PTs due to their exceptional diameter of 50 nm.