The dynamic pathway of nuclear RNA in eukaryotes

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Abbreviations: BR, Balbiani ring; EM, electron microscopy; FCS, fluorescence correlation spectroscopy; FISH, fluorescence in situ hybridization; FLIP, fluorescence loss in photobleaching; FRAP, fluorescence recovery after photobleaching; FRET, fluorescence resonance energy transfer; NPC, nuclear pore complex; ODN, oligodeoxynucleotide; PABP, poly(A) binding protein

The passage of mRNA molecules from the site of synthesis, through the nucleoplasm and the nuclear pore, en route to the cytoplasm, might appear straightforward. Nonetheless, several decades of detailed examination of this pathway, through the development of immuno-detection techniques and fluorescence techniques, to the current era of live-cell imaging, show this to be an eventful journey. In addition to mRNAs, several species of noncoding RNAs travel and function in the nucleus, some being retained within throughout their lifetime. This review will highlight the nucleoplasmic paths taken by mRNAs and noncoding RNAs in eukaryotic cells with special focus on live-cell data and in concurrence with the biophysical nature of the nucleus.

mRNA Visualized

Visualization of mRNA in living cells requires a means of fluorescently tagging the mRNA. Tagging of endogenous mRNAs was initially performed by hybridizing fluorescent probes to mRNAs. Early on, these experiments were performed with microinjected oligo(dT) probes that would hybridize with the poly(A) tail of the mRNAs, thereby generating a system of RNA fluorescence in situ hybridization (FISH) in living cells. Since it was not possible to differentiate by eye between the bound and the free probe, only techniques such as fluorescence correlation spectroscopy (FCS) or fluorescence recovery after photobleaching (FRAP) could be used to extract the kinetics of poly(A) populations in the nucleus. Later on, as GFP became an important player in time-lapse imaging experiments, a similar but more physiological approach was taken to label mRNA. Using GFP-poly(A) binding protein (GFP-PABP) or GFP-TAP, factors that are naturally harboring a different fluorophore, which hybridize to adjacent sequences on a cytoplasmic mRNA, it is possible to monitor energy transfer between the fluorophores and to perform measurements to detect specific cytoplasmic mRNA FRET signals.

Additional approaches have been devised, such as: microinjection into the nucleus of in vitro synthesized and fluorescence labeled mRNAs, complementation of split EGFP fragments fused to RNA-binding proteins that bind next to each other on the mRNA and thus reconstitute GFP fluorescence, or an aptamer coined “Spinach” that fluoresces upon binding of GFP derivatives. Many of these techniques have been recently discussed in detail. As for any scientific technology, no single approach is free of limitations. Specifically, the probe techniques typically require a rigorous method of delivery, such as microinjection, electroporation or streptolysin O permeabilization, and end with a step of hybridization that generates RNA-DNA duplexes, which is not trivial to accomplish when considering a live cell. Since mRNAs are coated with many proteins this would mean that the probes will have to compete with RNA-binding proteins and that certain portions of the RNA sequence will not be available for probe binding either due to protein binding or to secondary RNA structures. On the other hand the tagging of mRNA with GFP fusion proteins requires repeated sequences for multiple GFP-protein binding. This many times necessitates the insertion of RNA-binding sequences in tandem to be bound by many GFP-RNA binding proteins, adding exogenous protein components to the mRNP.
Many of these approaches do not enable the labeling of only a portion of the mRNA population or a specific mRNA species, not to mention the lack of single molecule sensitivity. To move the field forward it was necessary to overcome a major obstacle, namely how to efficiently tag the mRNA such that the signal-to-noise ratio is appropriate for imaging, leaving the background low enough for the detection of the mRNA molecules of interest. In a pioneer study, an oligo(dT) probe labeled with a chemically masked (caged) fluorescein molecule was used to tag the poly(A) RNA for visualization. Fluorescence of a portion of the mRNA population was achieved by irradiating the caged fluorescein using a 360 nm laser in only a small portion of the nucleus. This way the pathway of the fluorescent mRNAs could be highlighted as the un-caged signal dispersed within the nucleus, and indeed the measurements showed that mRNA motion is diffusion dominated.

Subsequently, single mRNA molecules could be followed using techniques that “light up” only the mRNA of interest and leave the background dark. For instance, molecular beacons that are administered to cells in a quenched fluorescent state and that only fluoresce once they are specifically hybridized with the mRNA. This technique was used successfully in many cell systems. A related approach uses hybridization of probes to cause the release of quenching of unique dyes and subsequent fluorescence. The use of molecular beacons could be somewhat tricky due to the requirement of probe opening and restructuring prior to hybridization, whereas the use of linear antisense probes is more straightforward but does not enable single molecule detection. The latter problem was overcome by multiply labeled tetrapodal RNA imaging antisense probes (MTRIPs) that have 4 or 5 fluorophores, which allowed the detection of single endogenous cytoplasmic mRNAs. This technique, however, requires the reversible permeabilization of the cells using streptolysin O.

The MS2 system, which is an RNA labeling method for the labeling of specific mRNAs, helped overcome some of the aforementioned challenges. The MS2 DNA sequence, when transcribed, forms stem-and-loop secondary structures that are recognized by an MS2 coat protein (CP) that binds as a dimer. By inserting the MS2 sequence in tandem in a gene of interest and simultaneously expressing the CP fused to a fluorescent protein, a system for tracking a specific mRNA in living cells is achieved. The repetitive nature of the MS2 sequences in the transcript, allows single molecule sensitivity by the binding of several GFP-MS2 proteins to the same mRNA. Similarly, using repetitive probes on a single mRNA sequence achieved single molecule detection. The MS2 system usually requires the transfection of a plasmid containing the gene of interest and thereby allows the overexpression of the mRNA, which is normally encoded by cDNA sequences lacking endogenous regulatory mRNA regions such as UTRs and introns. Therefore, the MS2 repeats have been useful mainly in studies that stably integrate a gene of interest into the genome. In the case of prokaryotes or lower eukaryotes that enable relatively easy genome integration procedures, it has been possible to associate the MS2 sequence repeats with endogenous genes, as demonstrated for bacteria, yeast, and Drosophila. The overexpression issue in mammalian cells was overcome by a stable integration approach that utilizes site-specific recombination and which culminates in the expression from a single allele only. This approach is complemented by the generation of knock-in mice containing the MS2 repeats in the 3'UTR of the endogenous β-actin gene, thereby allowing the studies of mRNA dynamics of endogenous mRNAs in the context of primary cells.

mRNA Tracked

Over the past two decades, fluorescence tagging was a key factor in the numerous attempts to unravel mRNA dynamics in the nucleus. Kinetic models applied to the data extracted from RNA FISH, FCS and FRAP experiments, enabled the calculation of mRNA diffusion coefficients and the categorization of the types of motion, such as free diffusion, corralled diffusion, or directed movement. Currently, two main approaches are applied to the study of mRNA kinetics, one being the analysis of the movement of a single mRNA particle by tracking and the other by monitoring the fluorescence recovery of a photobleached area. The former, combined with DNA labeling strategies, enabled researchers to characterize the nuclear routes of mRNA trafficking. In an early study, the nuclear distribution of the large Balbiani ring transcripts (~35 kb) from Chironomus tentans was analyzed using Br-UTP labeling and immune electron microscopy. The data showed that mRNAs move randomly in between chromatin with an estimated diffusion coefficient (D) in the range of 0.08–0.12 μm²/s. However, the nature of the recorded movement could not be decoded at the time (Brownian, corralled etc.) due to the use of fixed specimens. In another study in mammalian cells the diffusion rates of HCMV-IE mRNA were estimated to be 0.13 μm²/s.

The abovementioned RNA FISH techniques applied to living cells using oligo(dT)-fluorescently-labeled mRNAs set the stage for following the movement of the poly(A) population in real-time. The first measurements in living mammalian cells, using FCS and FRAP, revealed a range of diffusion coefficients between 0.01–10 μm²/s. One third of the diffusing oligo(dT)-poly(A) mRNAs were slow moving with average diffusion rates of ~0.39 μm²/s. This research, utilizing a caged fluorescein oligo(dT) probe to the poly(A) RNA population and observing the dissemination of the message, supported the notion of random movement in between chromatin domains, revealing a D value of 0.6 μm²/s for the labeled population. A similar D (0.6 ± 0.3 μm²/s) was found when poly(A) mRNAs were labeled with GFP-PABP2 in HeLa cells, but the data were later mathematically modeled and ended with a D value of 0.04 μm²/s. Upon energy depletion, a significant reduction in the movement of GFP-PABP2 was detected, implying that energy-dependent processes are involved in mRNA movement. Since these studies differ in diffusion coefficients values, a comparison between oligo(dT) and oligo(U22)-labeled poly(A) RNAs was conducted under identical conditions using FRAP analysis. Oligo(dT) probes were found to be highly mobile due to weaker affinity to poly(A) tails, which caused discrepancies in the measurements.
However, when a oligo(U)22 RNA probe was used, a D value of 0.04 μm2/s was found.

The range of diffusion coefficients obtained from poly(A) mRNA measurements suggests that different transcripts move at different rates. The development of the MS2 system54,55 and the subsequent identification of single mRNA molecules containing 24 MS2 sequence repeats56 allowed the examination of the movement of a specific mRNA molecules (mRNPs) by single particle tracking (SPT), for which a D value in the range of 0.04–0.05 μm2/s was measured.57 Thus, mRNAs were efficiently trapped by FRAP and photoactivation experiments. Diffusion governed mRNP movement and could be modulated as mostly correlated diffusion, probably due to obstruction of the pathway by chromatin and chromatin remodeling. This strengthened the notion that mRNA movement is random. Also, when a transcription site was observed near the nuclear membrane and in close vicinity to the NPCs, the mRNPs were seen dispersed throughout the nucleoplasm. The MS2 system was used to track specific mRNPs under ATP depletion conditions and it was found that mRNP retention due to chromatin restructuring is the reason for reduced mobility when using energy depletion treatments.58,59 Similarly, introducing an exogenous gene with molecular beacon target sequences enabled the tracking of a specific mRNA in hamster CHO cells. Two mRNP populations were found: mobile mRNPs which had a D of 0.03 μm2/s (free diffusion) and stationary mRNPs.60 The majority of studies employing the SPT methodology have used the mean square displacement model (MSD) to analyze the experimental data,61 and have shown that diffusion coefficients depend on the size of the mRNA.62–64 However, different approaches were tested as well.65 For example, jump-distance distribution analysis performed on the tracked Balbiani ring transcript revealed 4 poly(A)-RNA populations that differ in their diffusion rates. Further examination of the mRNA trajectories revealed that along their path, mRNPs sometime travel through more confined pathways, and sometime cruise through “open lanes,” concluding the same as MSD employed studies.66 Interestingly, although the nucleus of C. tentans is mostly nucleoplasmic and rather devoid of chromatin, mRNPs were found to move in a discontinuous fashion, suggesting interactions and stalling at sub-nuclear structures.67,68,69 Currently, the major technical difficulties in mRNA tracking are photobleaching of the fluorophore and the rapid movement in 3-dimensions. Imaging and tracking are typically performed in 2D, making it difficult to track mRNA movement from transcription site to cytoplasm. To tackle this issue, quantum dots, which are known for their fluorescence stability and strong intensity were used to track oligo(U) labelled poly(A) RNA in Cos7 cells for about 60 s using 30 μm/s intervals (D = 0.025 μm2/s). Although 60 s were not enough for full pathway coverage it is a step in the right direction.70

Tracking single mRNAs in the mammalian nucleus seems rather straightforward when thinking of performing this task in S. cerevisiae, since the diameter of budding yeast can be 5–6 μm, about half the diameter of a human or mouse nucleus. The yeast ARG3 housekeeping mRNA, which encodes the enzyme ornithine carbamoyltransferase, a non-localizing mRNA which is expressed at about 1–2 copies per cell, was a suitable candidate for tracking since only a few trajectories were expected thus reducing interference in a small space. The mRNA was tagged using 12 MS2 repeats integrated into the endogenous gene locus, thereby allowing expression by the endogenous promoter and the keeping of the original 3'UTR of the transcript. It was found that movement throughout the yeast cell could be categorized as random, confined or directed and diffusion coefficients were in the 0.039 μm2/s range as observed in other tracking studies.71

Single particle tracking has shown that mRNAs do not penetrate the nuclear membrane and in close vicinity to the NPCs, the mRNPs were not observed.72-74 Although mRNPs were not expected to be found in the nucleus, the question stands as to the biological mechanism behind this phenomenon. Single molecule analysis of fluorescent streptavidin molecules (60 kD) showed that these molecules accumulate significantly less in the nucleolus (60% less compared with the nucleoplasm), yet the nuclear border did not impede the access of the molecules, implying that the nucleolus is a highly permeable structure to molecules of this size.75 On the other hand, once inside, the movement was highly mobile. This was explained by measurements of nucleoplasmic vs. nucleolar density showing that there is substantially less free space in the nucleolus, therefore excluding molecules that do not have binding sites within the nucleolus. Nevertheless, streptavidin molecules are rather small compared with mRNP complexes and indeed exclusion of dextran molecules from the nucleolus has been shown to be size-dependent, as well as causing a significant reduction in diffusion in this dense compartment.76 The exclusion of mRNPs from nucleoli was directly visualized as the actual “bumping off” of single mRNP from nucleoli in live-cell movies.77 Moreover, during osmotic treatment nuclear regions become completely accessible to mRNP’s.78 mRNP molecules appear as rounded particles in time-lapse fluorescence microscopy movies, yet it is hard to infer structure from these images due to the resolution of light microscopy. Visualization of RNPs by electron microscopy indeed shows that these are compact and moderately elongated structures,79,80 and thereby can be regarded as particulate structures in analyses that model mRNP dynamics.58 A simulation of mRNA diffusion in the nucleus has shown that even if the mRNA wanders around the nucleus for a long time, it will find its way out.81 Also, regions of high density where an mRNA would have low diffusion coefficients would “push” mRNA toward the inter-chromatin domain that due to its small volume reduces the space in which an mRNA has to search until it finds an NPC. Altogether, this leads to efficient exploration of the inter-chromatin space and to rapid exit times.

**mRNAs Stuck in the Nucleus**

mRNAs are an easy target for tracking in living cells due to their size and our knowledge of the functional outcome inherent in them. Tracking of small RNAs, which are typically complexed with proteins to form small nuclear ribonucleoprotein particles, has been more challenging and challenging. For instance, fluorescently labeled U1 snRNPs, which are part of the core splicing machinery, were tracked at single molecule resolution in the mammalian nucleus and were shown to have diffusion coefficients in the range of 0.039 μm2/s as observed in other tracking studies.71
The mRNPs were observed to transiently bind to immobile cargo with short dwell times (50–350 ms), suggesting the detection of mRNPs involved in active splicing. The diffusion coefficient of fluorescein-tagged U7 snRNA was measured in the nucleoplasm of Xenopus oocytes and found to be 0.26 μm²/s. The behavior of mRNPs in nuclei of living cells was further elucidated using GFP-tagged splicing factors expressed at either exogenous or endogenous levels and following their kinetics using FCS and FRAP analysis. To obtain information for different snRNP species, this study showed that U1 and U4/U6 mRNPs have a dwell time of less than 1 s, whereas U2 and U5 mRNPs have longer interactions in the range of 15–30 s. Altogether, these results allow to estimate that the time-frame of the splicing reaction is around 30 s, although also longer times (0.5 min and 10 min) have been estimated. The mobility of snRNPs was found to be higher in nuclei of cells from spinal muscular atrophy (SMA) patients, a genetic disease that is caused due to low levels of the survival motor neuron (SMN) protein. This implies a reduction in the interactions of mRNPs with their nuclear binding sites and less incorporation into active spliceosomes, possibly leading to the widespread splicing defects associated with this disease.

Another example of a small nuclear retained RNA is found in the telomerase enzyme that functions in maintaining the length of telomere arms of linear eukaryotic chromosomes. Telomerase is comprised of several proteins and an RNA component, the latter serving as a template for the addition of telomere DNA sequences. Tagging of the yeast RNA component called TLC1 RNA with MS2 repeats enabled the tracking of telomerase in real-time. Contrary to the assumption that telomerase is stably associated with the telomeres, tracking of the telomerase particles in living cells showed a diffusive behavior similar to mRNPs. Following these dynamics throughout the cell cycle led to an interesting observation. During late S phase telomerase particles were larger and brighter than observed in G1 or G2 and the dynamics of these clusters correlated with the dynamics of the telomeric ends, implying a temporary stable association between telomeres and telomerase. Indeed, these structures were found to represent elongating telomerase complexes.

The expansion of CTG trinucleotide repeats in the 3'UTR of the dystrophin myostatin protein kinase (DMPK) gene causes myotonic dystrophy type I. Nuclear retention of the mutant mRNA is suggested to be a result of Mnbl1, a nuclear factor that facilitates protein transport. Still, as the NPC channel is considered a crowded environment, and the bi-directional flux of cargo is significant, there should be considerable competition for binding sites within the NPC. Therefore, this finding might reflect a given moment rather than the specialization of NPCs for the transfer of specific cargoes.

Where within the NPC do mRNAs translocate? Intriguingly, EM staining of various cargos has delineated two pathways of cargo movement through the NPC—one central path vs. a peripheral path (Fig. 1). This demarcation has been recently modeled in vitro, showing a central hydrophilic and electrostatically positive region that could act as an electrostatic barrier to positively
charged cargoes and as an attractant of negatively charged macromolecules with hydrophilic surfaces. A second more peripheral zone contains positively charged amino acid residues thereby attributing the passageway with a hydrophilic character. As for mRNA, RNA FISH in human cells showed that the staining was more intense in the pore periphery than in the middle, whereas gold labeling of mRNA showed central channel localization in *Xenopus* nuclear pores, as also observed in *C. tentans*. High-resolution analysis of this issue in human hematopoietic HL-60 cells and in rat liver tissue using Br-U labeled RNA and EM, as well as electron spectroscopic imaging (ESI), showed that mRNAs traffic via the periphery of the pore rather than through the central axis. In a study that determined the positions different cargo undertakes while passing through the pore of *S. cerevisiae*, the mRNA export factors Dhp5 and its partner Gle1 were centrally localized, thus suggesting that the later stages of mRNA export might be centrally located.

While EM experiments of mRNA staining have arrived at the conclusion that mRNA passage through the NPC must be very rapid, the time dimension was missing from these studies, and only recently was this issue tackled and demonstrated in living cells. Tracking large mRNAs expressed from exogenous genes in human cells showed the rather slow diffusion of mRNP through the nucleoplasm (time-scale of minutes), compared to the speedy exit from the nucleus on the range of less than 500 ms. High-resolution tracking of endogenous β-actin mRNAs in mouse cells has provided a breakdown of the timeframes for mRNP docking to the NPC and mRNP translocation through the pore and has found that the passage time of ~200 ms is spent mostly at the docking site at the nuclear basket and on the cytoplasmic filaments (although there was a subpopulation of the transcripts that spent ~2 s at the pore), whereas the dwell time in the pore was around 20 ms. This study suggests that there are unique docking and release steps for an exported mRNP. Recently, the export of the Balbiani ring (BR) mRNPs in living cells has been observed. The BR mRNP of *C. tentans* has served as a primary model for mRNA export due to its large size (32–40 kb) and thereby its detectability in EM sections.

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**Figure 1.** Following the pathway of mRNA in the nucleus. (A) mRNA labeling and tagging. Four main mRNA tagging methods are depicted in green: (1) Microinjection of in vitro synthesized and labeled mRNAs; (2) Hybridization of fluorescently-tagged DNA/RNA probes or molecular beacons (MB); (3) GFP fused-RNA binding proteins (GFP-PABP2 and GFP-TAP); and (4) MS2 tagging with the MS2-coat protein (CP). (B) mRNA tracking. Current methodologies for mRNA kinetic studies: single particle tracking (SPT), fluorescence correlation spectroscopy (FCS), and fluorescence recovery after photobleaching (FRAP). In yellow are the point of detection for FCS and the region photobleached during FRAP, and arrows mark the movement in and out of the spots. Fluorescent mRNPs are in green and photobleached ones are in black. (C) mRNA export. Left: the region in the pore through which mRNA (black dots) translocation occurs is represented by two paths (peripheral and central), as observed in EM studies. Right: the kinetic time-frame of mRNA movement through the nucleus and through the pore is described in *C. tentans* and mammalian cells. Chromatin regions appears in blue.
Seminal studies on native BR mRNPs led to the understand-
ing that the mRNA export process is directional since the 5' of the mRNA was observed to protrude first on the cytoplasmic side of the NPC. Moreover, in order to physically pass through the pore, the large mRNPs had to undergo a certain degree of unfolding or restructuring.95,96,97 Following BR mRNPs in live C. tentans salivary gland cells by use of microinjected fluorescently-labeled heps66, the homolog of mammalian hnRNP A1, and rapid imaging of the mRNPs with light-sheet fluores-
cent microscopy, demonstrated that export times could range between 65 ms and 6 s.98 This analysis could also detect differ-
ent modes of interaction: short interactions with the nuclear envelope suggested short-lived collisions with the NPCs, longer interactions that would agree with unsuccessful export events, and a relatively long binding step to the nuclear basket prior to effective export, which suggests that there is a rate-limiting step at the nuclear basket. Indeed, blocking of mRNA export in mammalian cells using a mutant form of the Dlp5 DEAD-box helicase supposedly required for release of export factors and restructuring of the mRNA during export, caused the docking step of mRNPs to be detected, as mRNPs remained bound to the nuclear envelope for long time periods due to the block,99 whereas such an occurrence is typically not observed due to the rapid kinetics of export.

Nuclear Structure and Dynamics Come Together

Chromatin regions, whether dense or open, have been shown to be relatively accessible to protein factors (even large ones) involved in replication, transcription and RNA processing. This was demonstrated by following the sub-nuclear distribution of microinjected, different-sized, fluorescent dextrans and large was demonstrated by following the sub-nuclear distribution of mRNPs in live C. tentans salivary gland cells by use of microinjectted fluorescently-labeled heps66, the homolog of mammalian hnRNP A1, and rapid imaging of the mRNPs with light-sheet fluorescence microscopy, demonstrated that export times could range between 65 ms and 6 s. This analysis could also detect differ-
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splicing of an mRNA are not sufficient for fruitful mRNA export.

Biophysics of the Nucleus

Assembling the different pieces of the puzzle together, we are now able to appreciate the emerging picture of nuclear biophysics. Studies with fluorescent dextran showed that diffusion in the nucleus is several fold slower than in water.128,129 Still, proteins move through the cell at high rates, as seen from bacterial "diffusion map."135 Using histone labeling by H2B-mRFP, the nucleoplasmic domain.115,133 The diffusion of molecules or complexes within the nucleus will be hindered by obstacles such as chromatin. Still, molecules in the nucleus must reach a certain destination, whether it is a transcription factor binding to a specific promoter sequence, a splicing factor attaching to a specific exon-intron junction in the pre-mRNA, or a mature mRNA traveling from the gene to the NPC. Since diffusion underlies the motion of molecules in the nucleus, it is supposed that the landscape of the nucleus, defined by the density of chromatin and nuclear bodies within, allows for the regulation of flow and the retention of molecules at specific sites of biological significance. A recent study using magnetic beads microinjected into the nucleus and followed at either 25°C or 37°C with or without magnetic force, has shown that the active remodeling of chromatin by ATP-dependent chromatin remodeling enzymes may drive molecule currents in the nucleoplasm.132 Another study using FCS to analyze the movement of EGFP, an inert molecule, throughout the nucleus established a "diffusion map."136 Using histone labeling by H2B-mRFP, the density of chromatin regions could be used to compare EGFP diffusion in different compartments. EGFP was homogeneously distributed and diffusion was found to be similar throughout the nucleus without any impediment by the chromatin network. Consequently, the actual traveling times of EGFP could be measured between two locations,137 in contrast to FCS that provides measurement of the molecules at one spot only without information of the pathway the molecule takes before or after entering the measurement spot. This analysis was performed with pair correlation function analysis (pCF) that measures anisotropic diffusion of EGFP molecules and showed that two types of flow could be detected that are influenced by the density of DNA in the region (density was measured using Hoechst 33342 DNA labeling). Because of the different DNA densities, a channel-like network appears in which EGFP can freely diffuse. However, flow or movement between the two compartments of either high or low density is restricted. Comparison to fluorescein, a much smaller molecule than EGFP, showed that diffusion of a small molecule was not obstructed in any way by high density chromatin. Interestingly, an infrequent burst-like movement of EGFP through the barrier between high/low density regions was also observed. This is a ~300 ms occurrence that suggests chromatin restructuring has taken place. Altogether, this approach demonstrates the organization of a channelled network of chromatin thereby directing molecules between different compartments through differences in DNA density.

Expanding our Vision of RNA Dynamics

As we delve deeper into the nuclear microenvironment, we find it is now possible to finally face old scientific problems with new technologies and the broadened perspective of nuclear processes. For instance, we long to understand how the large mRNA-protein complexes manage to travel efficiently through the NPC. While export is a rather fast process, large mRNPs may take longer due to size and the need for restructuring, as shown for the BR mRNP that undergoes structural changes as it squeezes through the NPC137 or for large mammalian mRNPs.138 Noteworthy regarding mRNA export, it has been suggested that the newly identified export pathway that does not use the NPC27 might be necessary for large cargos that do not pass through the NPC.126 and also resembles the egress of herpesvirus capsids from the nucleus.139 While we tend to think of RNA movement from nucleus to cytoplasm as a one way road,208 we should consider that the reverse is possible as well and might be physiologically relevant.139,140,141 Moreover, we know that cytoplasmic mechanisms can affect gene expression patterns in the nucleus by transducing forces via the proteins spanning the nuclear envelope.130-133,135,136 These forces might reflect on RNA dynamics too, as shown for nuclear body proteins.131,132 The dynamic properties of non-coding RNAs still await in-depth investigation, as is being performed for mRNA. While mRNAs typically display nuclear-cytoplasmic trafficking as a default mechanism, many non-coding RNAs are either nuclear retained or require a unique signal in order to facilitate translocation to the cytoplasm.141-144 Similarly, viral RNAs and their lifecycles also deserve renewed attention. For example, the polyadenylated nuclear (PAN) non-coding RNA is transcribed by RNA Pol II and is highly expressed during the lytic phase of Kaposi sarcoma-associated herpesvirus (KSHV) infection, during which it remains nuclear localized. Interestingly, during lytic KSHV infection the cytoplasmic poly(A)-binding protein C1 (PABPC1) relocates to the nucleus where it binds PAN RNA.145 In the case of PAN RNA, this RNA overloadsthe nucleus (500,000 transcript per nucleus), outnumbering by far the host and viral RNAs, as this species comprises ~80% of all polyadenylated RNAs during lytic infection. It remains to be seen what the function is of many of the coding and non-coding RNAs of viruses and how these affect the nuclear dynamics of host mRNAs either by direct interactions, titrating of RNA-binding proteins or overloading of the nucleus. Recently, another mRNA tagging system based on repetitive sequences has been implemented in mRNA quantification and live-cell imaging experiments. Similar to the MS2 system, the PP7 tag is based on an RNA hairpin sequence and its cognate binding protein from the Pseudomonas aeruginosa PP7 bacteriophage.144 Currently, this tag has been used together with the MS2.
malian cells. Further utilization of the PP7 system, which may be even better than the MS2 system in mammalian cells, will be worthwhile for broadening our capacity to visualize and correlate between two mRNA species in the same cell.

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