RNA imaging: seeing is believing

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Twenty years ago, in the middle 1990s, a pending debate in the RNA cell biology field concerned the nuclear organization of pre-mRNA splicing. Localization studies using antibodies to detect proteins of the spliceosome and oligonucleotide probes that hybridized with the spliceosomal small nuclear RNAs had revealed that practically all building blocks of the splicing machinery were not uniformly distributed in the nucleus but rather appeared concentrated in defined regions. These regions were termed "nuclear speckles" or "splicing domains" due to the local enrichment in splicing factors. Whether or not pre-mRNA splicing occurred within these domains remained controversial. To address this question, it was critical to visualize the transition from nascent unspliced transcripts to the spliced forms. This was made possible through successive optimizations of in situ hybridization, a microscopic technique pioneered independently by Gall and Jones back in the late 1960s. An important advance was made by Lawrence, who introduced a procedure for fluorescent detection of nuclear RNA using biotin-labeled DNA probes and fluorophore-conjugated avidin. During the 1990s, the method was further optimized for the simultaneous detection of DNA, unspliced precursors and spliced mRNA. Using these tools, several laboratories showed that spliced mRNA localized in close proximity to the DNA from which it was transcribed, consistent with earlier electron micrographs obtained by Beyer and Osheim suggesting that the spliceosome formed shortly after synthesis of the 3′ splice site and that splicing of pre-mRNA could occur while the pre-mRNA was still tethered to the gene locus via RNA polymerase.

A subsequent imaging breakthrough resulted from the development of genetically encoded fluorescent tags that, combined with fluorescence-based microscopic approaches of increasingly higher spatial and temporal resolution, made it possible to analyze protein movement in living cells. Tom Misteli in the Spector lab reported the first time-series recordings of cells expressing the green fluorescent protein (GFP) fused to an essential splicing protein. These studies provided direct evidence that nuclear speckles supply spliceosomal components to nearby activated sites of transcription and splicing. Further developments of FRAP (fluorescence recovery after photobleaching) methods unravelled the kinetic properties of splicing proteins in the nucleus of live cells. FRAP studies showed that spliceosomal proteins are continuously moving in the nucleus, shuttling in and out of nuclear speckles within seconds. Since actively transcribed genes are intimately associated with the periphery of nuclear speckles, one possibility is that spatial confinement of spliceosomal components in dedicated compartments increases their local concentration in the neighborhood of nascent transcripts, thus enhancing spliceosome assembly on newly synthesized introns. This could be a reason why splicing is so much faster in vivo than in vitro. FRAP analysis further indicated that the trafficking kinetics of splicing factors in and out of nuclear speckles is independent of ongoing transcription and splicing, arguing that spliceosomal components are not stored in nuclear speckles until a signal triggers their recruitment to nascent introns; rather, it is more likely that components of the spliceosome are constantly roaming the nuclear space until they collide and transiently interact with either a nascent pre-mRNA, to form a spliceosome, or with other splicing proteins at nuclear speckles.

In 2004, two seminal reports from the Singer and Spector labs revolutionized the RNA imaging field by pioneering an approach to visualize mRNA synthesis and transport in living mammalian cells. The method consists in genetically inserting binding sites for the MS2 bacteriophage coat protein in a gene of interest. The resulting reporter transgene is then integrated in the genome of mammalian cells that express the MS2 coat protein fused to a fluorescent protein such as GFP. Upon transcription, binding of fluorescent coat protein to its target sequence makes that particular mRNA visible in living cells. With this approach it was possible to track, in real-time, the life cycle of an mRNA tagged in the 3′ UTR, from transcription to transport and localization in the cytoplasm. The results showed unequivocally that mRNAs diffuse throughout the nucleus and hit nuclear pores in a stochastic manner. This came as a shock to many cell biologists that advocated the existence of an internal nuclear scaffold (or "matrix") composed of a fibrillar system that would

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guide mRNAs from the site of transcription directly to the pores.

Another surprising revelation of RNA imaging studies was the occurrence of random fluctuations in transcription of individual genes. Using either the MS2-GFP system or variants of fluorescence in situ hybridization (FISH) methodologies capable of detecting individual mRNA molecules in single cells, different laboratories demonstrated that many genes in both prokaryotes and eukaryotes are transcribed in a pulsatile fashion. Potential biological implications of such transcriptional bursts are just starting to be experimentally explored. While accuracy in all steps of gene expression is vital for cellular and organismal integrity, stochastic fluctuations are likely important for biological plasticity. To date, many studies link stochastic gene expression with cell-fate decisions during development of higher eukaryotes. There is also evidence that aging is correlated with increased noise in gene expression. A fascinating line of future research will be to determine the molecular mechanisms involved in cell-to-cell variability in gene expression patterns and to understand how cells control and tolerate noise in each step of gene expression, from transcription to pre-mRNA processing and translation. This will likely rely on the development of new imaging strategies capable of monitoring in real time the dynamic behavior of single endogenous molecules in living cells and tissues. If randomness plays a fundamental role in the establishment of gene expression patterns, as anticipated, future discoveries on this topic may prove important in ways that are unpredictable today.

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