Trends in Genetics

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Review

Dynamic Encounters of Genes and Transcripts with the Nuclear Pore

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Transcribed mRNA molecules must reach the cytoplasm to undergo translation. Technological developments in imaging have placed mRNAs under the spotlight, allowing the quantitative study of the spatial and temporal dynamics of the nucleocytoplasmic mRNA export process. Here, we discuss studies that have used such experimental approaches to demonstrate that gene tethering at the nuclear pore complex (NPC) regulates mRNA expression, and to characterize mRNA dynamics during transport in real time. The paths taken by mRNAs as they move from their sites of transcription and travel through the nucleoplasm, in between chromatin domains, and finally through the NPC, can now be observed in detail.

Trends

High-resolution microscopy enables real-time measurements of nuclear mRNA transport in different cell types, including the timing of mRNA transport to the pore and the differences in nuclear dwell times between transcripts, some of which may remain in the nucleus for many hours.

Using imaging techniques, it is possible to detect separate scanning and docking events of mRNAs at the NPC, and to measure the rapid steps of export per se at the nuclear basket, in the channel, and before release on the cytoplasmic side. Even rare bidirectional transport events of mRNA can be observed.

Regulation of gene expression at the pore can be governed through tethering of genomic regions to the pore via interactions with nucleoporins and transcription factors. This can lead to either induction or repression of transcriptional activity of genes repositioned at the nuclear periphery.

Visualizing the Nuclear Pathway of an mRNA Molecule

mRNA transport and export follow a general trajectory from the nucleus to the cytoplasm. However, the actual movement of mRNA in the nucleus relies on a random diffusion-based mechanism [1–10]. Studies in mammalian, yeast, and *Chironomus tentans* cells, the latter a nice model for detecting large mRNAs by electron microscopy (EM) [11], have unraveled the dynamics of single mRNA molecules traveling within the nucleus and caught in the act of nucleocytoplasmic transport (reviewed in [12–14]). Developments in microscopy in both fixed and living cells have allowed the detection and tracking of single mRNA molecules on their journey from their site of synthesis, through the nucleoplasm, in between chromatin regions, to their docking sites at the NPC, culminating in transport into the cytoplasm. Techniques for labeling RNAs either by hybridization with fluorescent probes or by indirect tagging with fluorescent RNA-binding proteins have taken over the field, filling in where EM is lacking (Box 1). It is now possible to examine the transport timeframe stepwise from transcription to the NPC. These advances have led to: (i) the determination of how gene positioning in the nuclear volume and in the vicinity of the NPC affects expression in different organisms; (ii) the detection of mRNA docking and scanning at the NPC; (iii) the determination of whether there are queues of mRNAs at the NPC waiting to pass through; (iv) the measurement of the timescale of export per se through the NPC; and (v) the determination of what happens to the mRNA on the cytoplasmic side of the NPC.

Encounters between Genes and NPCs

Increasing findings over the years point to mechanisms of gene tethering at NPCs, which are assumed to generate chromatin hubs that regulate gene activity in either a positive or negative manner. The 'gene gating' hypothesis proposed three decades ago by Gunther Blobel suggested that active genes located proximal to NPCs would preferentially export their mRNAs into the cytoplasm using the NPCs to which they were gated [15]. Historically, the nuclear periphery has been considered a gene-silencing zone, containing heterochromatin or gene-poor parts of

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Box 1. Tagging mRNAs in Living Cells

Detection of mRNAs in intact cells began with EM approaches, either by direct observation due to the large size of the mRNP studied (e.g., the large Balbiani Ring mRNPs in *Chironomus tentans* [111]), or via *in situ* hybridization [e.g., radioactive/dye-labeled probes that bind to polyA(+) mRNAs [60–62]]. This led to the development of the now popular RNA fluorescent *in situ* hybridization (FISH) technique in which a series of fluorescent probes that hybridize to a specific mRNA enable the detection of mRNA molecules in cells and tissues [112]. Moving these studies from fixed to living cells required new technical approaches. Hybridization can be performed in living cells by microinjecting fluorescent probes directly into single cells. For instance, the intranuclear mobility of the polyA(+) population was first measured using a fluorescent oligo(dT) probe that hybridizes with all polyA-containing RNAs [3,4,10]. Later, fluorescent versions of RNA-binding proteins that can bind to all mRNAs were used to label the endogenous RNA population in living cells [6,8,9]. The detection of a specific mRNA species required the insertion of a unique tag sequence into the mRNA of interest, establishing the MS2-tagging technique [113]. This approach inserts a repeated series of MS2 stem-loops into the mRNA, which are specifically bound by a series of GFP-MS2 coat proteins (GFP-MCP), thus allowing the detection of single molecules of mRNA in living cells [1]. The logic of using tandem repeats of a tagging sequence within the mRNA as multiple binding sites for fluorescent probes was also implemented for the hybridization of molecular beacons that only fluorescence upon hybridization to the mRNA [7,49]. Most of these approaches enabled the tracking of exogenous mRNAs to which the tagging sequences were cloned, but MS2-tagging has now also been implemented for endogenous mRNAs [114,115]. Recently, the utilization of additional types of stem-loop tag has expanded the options for either the detection of several RNA species simultaneously or discriminating between different regions of the mRNA, namely introns and exons [108,116,117].

the genome. However, this is probably not entirely the case, as shown by the targeting of active genes to the NPCs in *Saccharomyces cerevisiae* [16–19] and *Caenorhabditis elegans* [20]. Still, some genomic associations with NPCs, for instance Nup170 in yeast, confer repressive signals [21].

Many of the yeast genes identified as being activated at the NPC function in metabolic pathways requiring rapid responses to changes in nutrients in the environment [16]. The functional significance of the association of active genes with the nuclear periphery is debatable. This phenomenon might be consistent with a ‘gene gating’ model in which the diffusive passage of mRNA into the cytoplasm is facilitated by proximity to the pore (Figure 1). Rather than waiting for mRNAs to slowly diffuse in the nucleus until they find the point of exit, particularly in cells such as yeast, which might require a quick response to a changing environment, the site of transcription serves also as the site of export. Thus, gene expression kinetics would be enhanced. However, the yeast nucleus is rather small and recent studies have shown that mRNAs are rapidly shunted out to the cytoplasm [22], arguing against the need for the cell to generate a ‘super-fast’ track out. Also, mammalian mRNAs transcribed from a gene situated in close proximity to the nuclear envelope diffused throughout the whole nucleus and did not use the shortest exit route [1].

Hence, the emerging paradigm is that genes are targeted to NPCs, in some cases via unique ‘zip code’ sequences (Box 2), and attach through interactions with nucleoporins (Nups) and transcription factors. A recent study in yeast suggested that different gene families are recruited by different types of transcription factors, thereby adding layers of regulation to this process [23]. Therefore, gene association with the NPC enables transcriptional regulation and a switch-like transcriptional response for gene loci, interchanging between active and repressed states (Figure 1). For instance, some inducible genes can form DNA loops at the proximity of the NPC, allowing a faster re-induction after repression, such that these interactions serve as a ‘memory’ for future cycles of transcription, rather than for expedited mRNA export [24–26]. A tethering mechanism in *C. elegans* has also been identified for noncoding RNAs, namely, small nucleolar RNAs (snoRNAs) transcribed by RNA polymerase III, most probably with the Pol III pre-initiation complex forming inside the nuclear pore [27]. Noteworthy with respect to transcriptional control, is that the influence of the NPC components on gene activity runs beyond mRNA transport, because it is well established that some Nups have a nucleoplasmic role away from the pore, such that they influence transcription levels either directly by binding to promoters or indirectly by affecting chromatin structure [28].

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