RNA Travel: Tracks from DNA to Cytoplasm

Minireview

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Is There a Spatial Component to Nuclear RNA Processing and Transport?

A controversial model holds that the RNA processing machinery resides at defined locations in the nucleus and that RNA transcripts travel on defined paths through these sites on their route to the cytoplasm. Thus, transcription, processing, and transport into the cytoplasm would occur in an ordered fashion as transcripts move from the gene to the nuclear pores. The spatial features of this model are analogous to the movement of a newly synthesized polypeptide through the cytoplasmic secretion machinery. The processing machinery (signal peptidase, glycosyl transferases, etc.) resides at defined locations, and the polypeptide to be secreted is transported from place to place (Pryor et al., 1992).

This transport model makes two predictions. First, the components involved in these nuclear processes, e.g., active RNA polymerase II, splicing components, and nuclear pores, should have different and defined locations, perhaps sequentially distal from the location of the genes. Second, the steady–state distribution of nuclear transcripts (in flagrante transporto) might be nonuniform, reflecting a defined route between processing stations or between the active gene and the nuclear periphery. These predictions are supported by some studies, but also contradicted by other studies. We present both sides of this interesting controversy in the hope of stimulating critical thinking about how RNA is transported from its site of synthesis to the cytoplasm.

Speckles, Foci, and Tracks

In support of the prediction that nuclear components reside at defined locations is the notable concentration of splicing components in a number of discrete light microscopic subnuclear domains, termed speckles and foci (e.g., Spector et al., 1991; Carmo-Fonseca et al., 1992). Although different in a number of ways, both nuclear structures have been previously defined at the electron microscopic level; the larger, more intense speckles are probably interchromatin granules, and foci are coiled bodies. In support of the prediction that transcripts follow a defined route, is the description of nuclear transcript tracks or pathways from an Epstein–Barr virus-transformed cell line (Lawrence et al., 1989) or from a serum-induced c-fos gene (Huang and Spector, 1991). Taken together, it is tempting to draw a simple cell biological picture: pre-mRNA is spliced in speckles (and perhaps also in foci), and then the fully processed transcripts are transported along tracks

to the nuclear pores, where they exit to the cytoplasm. Consistent with this view is the fact that intron-containing RNAs are targeted to speckles upon microinjection into mammalian nuclei (Wang et al., 1991).

Only Some Genes Make Tracks?

This structural view has received further support from two recent papers (Xing et al., 1993; Carter et al., 1993). The techniques of digital imaging microscopy were used to examine at high resolution the subnuclear domains that contain individual transcripts, splicing components, and poly(A) RNA (the latter referred to as transcript domains). Two specific RNAs, fibronectin and neurotensin, were examined; the intron and exon portions of the fibronectin transcript were individually characterized and the subnuclear localizations compared with that of the corresponding gene, splicing factor SC-35, and general poly(A). Both specific transcripts were characterized by one or two sites of hybridization nearly coincident with the gene, indicating that the highest concentration was at or adjacent to the site of transcription. This was first observed by Shermoen and O'Farrell (1991), who showed that nuclear transcripts in early Drosophila embryos colocalize with their genes.

A more detailed examination of the fibronectin transcripts gave rise to two principal observations. First, fibronectin exon sequences frequently accumulated in tracks similar to the EBV and c-fos results. Second, intron-containing RNA was less track-like and more restricted toward one end of the exon-containing track. Although only exon sequences were colocalized with the gene, the intron RNA-containing end is presumably proximal to the gene. One might extend these observations to suggest that splicing of the fibronectin primary transcript takes place shortly after transcription (the gene signal was more restricted than that of the intron-containing RNA) and that only spliced RNA is transported along a track toward the nuclear periphery.

On the other hand, when the neurotensin gene was examined, tracks were not seen. Although difficult to evaluate in the absence of quantitation, this disparity might have been exaggerated by differences in gene size or exon number; e.g., the fibronectin gene is three times the size of neurotensin. Another technical difficulty is that in situ hybridization is only capable of comparing steady-state levels between (accessible) RNA sequences. Since the steady-state distributions of fibronectin and neurotensin nuclear transcripts have not been biochemically described, the rate-determining steps and lifetimes of the individual molecular species are not known. For example, the intron signal at the gene could be due to free introns as well as to intron-containing pre-mRNA. The same consideration applies to the observation that few if any of these tracks appear to reach the nuclear envelope; perhaps a rapid transport or a slow processing step accounts for apparent termination of the track short of the pore.

In a different and biochemically well-characterized system, Zachar et al. (1993) describe experiments that failed

to support a tracked route of RNA transport through the nucleus. This study, which examined the intranuclear distribution of a highly expressed chimeric transcript in the salivary glands of Drosophila third instar larvae, indicated that the intense signal was likely due to nascent RNA. The pictures were especially revealing, as the strong transcription of this gene gave rise to intense local hybridization. In addition, considerable specific fluorescence was evident throughout much of the rest of the nucleus, which appeared restricted to a web-like pattern. Although insect polytene nuclei are unusual, the observations suggest that a diffusion-mediated process through a channel network may govern RNA movement from a gene to the nuclear periphery.

Colocalization of Tracks and RNA Processing Components?

A further prediction of the spatial organization model is that there should be colocalization of the tracks and some identified, functional component of the processing system. By hybridization with poly(dT) to localize poly(A) RNA, Carter et al. (1993) found that transcript domains contain high concentrations of the splicing factor SC-35, i.e., that transcript domains and speckles coincide or nearly so. In Xing et al. (1993), individual transcript domains were also shown to be close to the actively transcribing genes (see also Huang and Spector, 1991). The implication is that the poly(A) signal is due to recently synthesized pre-mRNA, located at a processing (splicing) station and on its way to the cytoplasm. However, the steady-state nature of the in situ hybridization technique does not indicate direction or velocity, suggesting that some other approach should be taken to support the contention that the poly(A) RNA in the transcript domains is in transit to the cytoplasm. As classical biochemical experiments show that newly synthesized nuclear RNA is rapidly transported to the cytoplasm during incubation with actinomycin D (e.g., Penman et al., 1970; Rosbash, 1972) and that nuclear poly(A) is conserved during nucleocytoplasmic transport (Nevins and Darnell, 1978), an in situ hybridization chase experiment with transcriptional inhibitors should be revealing.

There are recent additional observations incompatible with the idea that these domains contain newly synthesized transcripts on their way to the cytoplasm. Using antibodies to visualize bromouridine incorporation, two papers suggest that nascent RNA is located into a large number (200–500) of domains dispersed throughout the nucleus (Jackson et al., 1993; Wansink et al., 1993). As the authors note, this organization is different from that observed for speckles. Even with longer labeling times (designed to follow polymerase II transcripts from their sites of synthesis to their subsequent destinations), there is no further colocalization of the bromouridine-containing transcripts and the sites of intense SC-35 staining. The observations are consistent with earlier work that used 3H-uridine labeling to define perichromatin fibrils as sites of active transcription (Fakan et al., 1976; Fakan and Nobis, 1978).

The hypothesis that many (perhaps most) splicing events take place outside of the speckle/transcript domains has some indirect support from experiments per-

formed in the yeast, Saccharomyces cerevisiae. In this organism, splicing components and splicing mechanisms are likely to resemble closely those in metazoan organisms (Rymond and Rosbash, 1992). There is even some evidence that the nuclear organization is similar, i.e., speckles or speckle-like subnuclear domains have been described (Elliott et al., 1992). In the relevant experiments, the concentration of splicing components (including U1, U2, and U5 snRNPs) was reduced in vivo by genetic depletion strategies (Patterson and Guthrie, 1987; Séraphin and Rosbash, 1989). The experiments suggest that there is between 10-fold and 100-fold excess of U1 snRNP over what is required for wild-type splicing efficiency and growth rates (Séraphin and Rosbash, 1989). Thus, speckles could be storage sites for splicing components, rather than being directly engaged in the splicing of newly synthesized pre-mRNA (e.g., Jiménez-García and Spector, 1993). A precedent for the storage of splicing factors in defined morphological structures is provided by the description of snurposomes in Xenopus oocytes (Gall, 1991). It is possible, therefore, that much pre-mRNA splicing takes place in the regions of the nucleus associated with active transcription but relatively low splicing factor concentration, i.e., the perichromatin fibrils (Spector et al., 1991).

Are Tracks Completed or Nascent RNA?

All of these considerations suggest that a more comprehensive model may be required to accommodate these diverse observations. If the track contains mainly completed transcripts, then either diffusion (possibly constrained by chromatin or matrix components) or the relative rate of different active transport steps could account for its dimensions. For example, a slow step (interaction with a transporter?) might cause a queuing of polyadenylated RNAs (partially or fully processed) waiting for letters of transit. Another possibility is that the tracks are high concentration sites of nascent RNA, tethered at the 3' end to the transcriptional machinery. As there is evidence for cotranscrip-
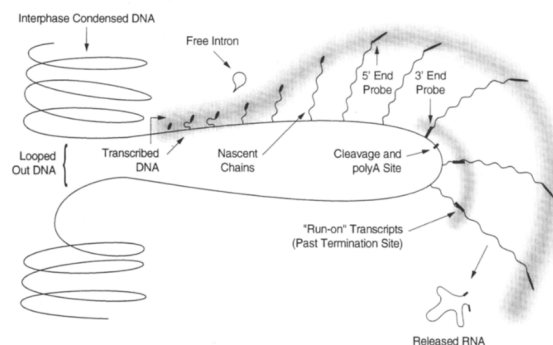

Figure 1. Transcription and Processing of a Hypothetical Gene

Transcription begins at the base of the loop and proceeds toward the apex. A 5' intron-containing probe would visualize the transcripts only near the condensed DNA. A 5' exon probe would visualize the entire length of the gene because it would hybridize to all transcripts, whereas a 3' exon probe would visualize only the later transcripts at the distal end of the gene (stippled areas). Released RNA would hybridize to both probes in stoichiometrically equal amounts. Run-on transcripts may progress past the cleavage–polyadenylation site, depending on the rate of the cleavage step. After release, RNA may persist near the gene before diffusion or active transport (see text).

tional splicing (Beyer and Osheim, 1988; LeMaire and Thummel, 1990), this suggests that the 5' part of the track might be intron-free whereas the 3' end would be collinear with the gene. For nascent RNA, the track length would be influenced by a number of variables, as illustrated in Figure 1. These variables include the length of decondensed chromatin, the length of the primary transcript (condensed in vivo by an unknown factor), and the efficiency of the RNA processing events. For example, the rate of 3' cleavage and polyadenylation is likely to be important, as transcription may proceed for some distance past the polyadenylation site. These considerations suggest that nascent transcripts might make a significant length contribution to a track and that the rates of the individual processing reactions are likely to impact the in situ hybridization picture in a gene-specific fashion.

Although speculative, the nascent chain-track hypothesis has the virtue of being easily tested: hybridization with probes that come from the 5' and 3' ends of a large gene should give rise to different patterns, quantitatively and qualitatively. The preponderance of signal should be from the 5' end probe, which might coincide with the 3' end probe only at the distal end of the transcription unit. Results from these types of approaches should help resolve this key question: how does RNA travel from its site of transcription to the cytoplasm?

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