Discrete nuclear domains of poly(A) RNA and their relationship to the functional organization of the nucleus

Kenneth C. Carter  
*University of Massachusetts Medical School*

Krishan L. Taneja

Jeanne B. Lawrence  
*University of Massachusetts Medical School, Jeanne.Lawrence@umassmed.edu*

Follow this and additional works at: [http://escholarship.umassmed.edu/oapubs](http://escholarship.umassmed.edu/oapubs)  
Part of the [Cell Biology Commons](http://escholarship.umassmed.edu/oapubs), and the [Medicine and Health Sciences Commons](http://escholarship.umassmed.edu/oapubs)

**Repository Citation**
[http://escholarship.umassmed.edu/oapubs/962](http://escholarship.umassmed.edu/oapubs/962)

This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in Open Access Articles by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.
Discrete Nuclear Domains of Poly(A) RNA and Their Relationship to the Functional Organization of the Nucleus

Kenneth C. Carter, Krishan L. Taneja, and Jeanne Bentley Lawrence
Department of Cell Biology, University of Massachusetts Medical Center, Worcester, Massachusetts 01655

Abstract. The functional organization of the nucleus was studied using a fluorescence microscopy approach which allowed integration of positional information for RNA, DNA, and proteins. In cells from sea urchin to human, nuclear poly(A) RNA was found concentrated primarily within several discrete "transcript domains" which often surrounded nucleoli. Concentrations of poly(A) RNA were coincident with snRNP antigen clusters, providing evidence for the localization of pre-mRNA splicing at these sites. The spatial relationship of transcript domains with respect to various classes of DNA was established, in that the poly(A) RNA-rich regions coincided with discrete regions of low DNA density and were non-randomly distributed with respect to specific DNA sequences. Centromeric DNA and late-replicating DNA did not overlap transcript domains, whereas a subset of early-replicating DNA may. Results indicate that transcript domains do not result directly from a simple clustering of chromatin corresponding to metaphase chromosomes bands. Finally, observations on the reassembly of these domains after mitosis suggest that the clustering of snRNP antigens may be dependent on the reappearance of pol II transcription. Implications of these findings for overall nuclear structure and function are considered, including a discussion of whether transcript domains may be sites of polymerase II transcription reflecting a clustering of active genes.

The cell nucleus performs numerous complex tasks which include packaging the enormous length of DNA, high fidelity replication of this DNA, transcription, processing, and transport of different classes of RNA, protein import and export, and precise redistribution of nuclear components during mitosis. Additionally, many of these are done in a cell-type specific manner. It has often been proposed that the extremely dense structure of the nucleus may be spatially compartmentalized as a means to efficiently carry out its multiple functions (see Comings, 1968, 1980). However, evidence for such physical partitioning has been modest. For example, while great advances have been made in understanding the biochemical steps involved in pre-mRNA transcription and processing, the subnuclear location of these events and the extent to which they are compartmentalized is unknown. In this paper we investigate the potential compartmentalization of these and other nuclear events by analyzing the distribution of poly(A) RNA in relation to several nuclear constituents of known functional significance.

Unlike the cytoplasm, there are no lipid membranes in the nucleus, but the confinement to the nucleolus of rRNA genes originating on several different chromosomes shows that elegant spatial and functional regionalization is possible. Strong evidence supporting the early suggestions that interphase centromeres and telomeres are specifically positioned (Rabl, 1885) and individual chromosomes occupy distinct territories (Boveri, 1909) has been presented recently by several laboratories (Reviewed in Hadlaczky et al., 1986; Manuelidis, 1990; Haas and Schmid, 1991). While most studies have focused on the position of entire chromosomes or abundant non-expressed sequences, visualization of a single gene and its cognate RNA suggests that the interphase positions of specific active sequences and their primary transcripts may also be highly localized (Lawrence et al., 1988; Lawrence et al., 1989). That interphase chromatin may be functionally as well as spatially compartmentalized is also implied by the fact that metaphase chromosomes display unique and highly reproducible patterns of light and dark bands with respect to which genes (reviewed in Bickmore and Sumner, 1989), replicating DNA (Ganner and Evans, 1971; Holmquist et al., 1982), repetitive sequences (Manuelidis and Ward, 1984; Koenberg and Rykowski, 1988), nuclease sensitivity (Gazit, 1982; Kerem, 1984), and certain proteins (Disney et al., 1989) are specifically positioned. It is quite possible that these bands correspond to some distinct functional partitioning of chromatin at interphase.

Additional evidence for higher level nuclear organization comes from immunolocalization studies which established early on that nuclear antigens recognized by serum from autoimmune disease patients can be categorized as either homogeneous, nucleolar, or "speckled" (Beck, 1961). Despite extensive investigation of autoimmune antigens, the reasons for many of them having nonhomogeneous nuclear localization are not known. Some autoimmune antibodies selectively precipitate small nuclear ribonucleoprotein particles.
Materials and Methods

Cell Culture

Human diploid fibroblasts, originally cultured from the foreskin of a normal male newborn (Folkman and Haudenschild, 1980) were grown as monolayers in DME plus 0.1% glucose and 10% FCS (Gibco Laboratories, Gaithersburg, MD) and purified using a G-50 Sephadex column (Taneja and Singer, in preparation). Hybridizations were done at 37°C in our standard conditions. Likewise snRNP antigens remained intact during NaOH treatment, as determined by immunofluorescence, further suggesting that the loss of T55 signal was due specifically to the removal of RNA. Areas of low T55 signal, for example near the nuclear envelope and in early G1 cells, do not result from low accessibility to probe molecules since centromeres were readily detected here.

Immuno-fluorescent Staining of snRNP Antigens

The snRNP mAb used, Y12, is categorized as an anti-SM Ab because it selectively precipitates ribonucleoprotein complexes containing U1, U2, U4, U5, and U6 RNAs, all of which are involved in pre-mRNA splicing (Lerner et al., 1979, 1980, 1981; Yang et al., 1981). Although not universally accepted, there is evidence that antigens from this class of snRNPs are clustered primarily in certain nuclear regions in mamalian somatic cells (Mattioli and Reichlin, 1971; Northway and Tan, 1972; Lerner et al., 1981; Deng et al., 1981; Tan, 1982; Spector et al., 1983; Fakan et al., 1984; Reuter et al., 1984; Smith et al., 1985; Nyman et al., 1986; Ringertz et al., 1986; Spector, 1990; Zieve and Sauterer, 1990) and in the sphere organelle in amphibian oocytes (Gall and Callan, 1986; Wu et al., 1991). A spliceosome assembly factor also localizes to these areas (Fu and Maniatis, 1990), but it remains to be shown unequivocally whether these regions exist in vivo, and if so whether they are sites of pre-mRNA processing or, alternatively, sites of snRNP assembly or storage, with pre-mRNA processing occurring elsewhere (Meadows, 1990; Fu and Maniatis, 1990; Spector, 1990; Zieve and Sauterer, 1990).

A well-integrated structural and functional view of the nucleus will require approaches which simultaneously localize, with high resolution, specific functionally distinct nuclear constituents, and which ultimately consider the distribution of nuclear RNA as well as DNA and proteins. Using such an approach we have studied the intranuclear distribution of poly(A) RNA because of its fundamental significance for understanding the spatial organization of pre-mRNA transcription, processing, and transport. Approximately 90% of mRNA is polyadenylated and essentially all nuclear poly(A) sequence occurs at 3′ tails on hnRNA destined to become cytoplasmic message (reviewed in Lewin, 1975; Puckett and Darnell, 1976; Brawerman, 1981; Nevins, 1983), therefore the distribution of this broad category of RNAs can be investigated by fluorescent in situ hybridization to their poly(A) sequences. Taking this strategy we demonstrate that poly(A) RNA is concentrated in discrete domains within the nucleus. The positions of these “transcript domains” were compared in individual cells with locations of the nucleolus, total DNA, replicating DNA, centromeric DNA, and RNA processing components. The potential relationship of this distinct nuclear compartment to chromosome organization was also considered as was the reassembly of these areas following mitosis.

In Situ Hybridization

Hybridization and fluorescence detection were based on previously developed methods described in detail elsewhere (Lawrence and Singer, 1985; Lawrence et al., 1988, 1989; Johnson et al., 1991b). Oligo dT55 (T55) was end labeled with biotin-16-dUTP (Bethesda Research Laboratories, Gaithersburg, MD) and purified using a G-50 Sephadex column (Taneja and Singer, in preparation). Hybridizations were done at 37°C in our standard buffer and 15% formamide (Johnson et al., 1991b). Detection was done using fluorochrome-conjugated avidin (Enzo Biochemical) in 4x SSC.

Hybridization Controls

To establish that T55 signal resulted from specific hybridization, parallel experiments were done replacing oligo dT with dA (see Fig. 1), dC, or dG (not shown), each of which resulted in no signal. When cells were hybridized with biotin- or 32P-labeled T55 followed by subsequent washes at stepwise increases of 5°C (starting at 35°C), >95% of the signal was lost between 45 and 50°C, indicating the sharp melting curve of oligonucleotide hybrids. Excess nonlabeled T55 or A55, but not a random sequence 55 mer, inhibited binding of labeled T55. When fixed cells were treated before hybridization with 0.2 N NaOH to remove cellular RNA, no T55 hybridization occurred. This was not because of inhibition of hybridization or detection since centromeric DNA was readily detectable under identical conditions. Likewise snRNP antigens remained intact during NaOH treatment, as determined by immunofluorescence, further suggesting that the loss of T55 signal was due specifically to the removal of RNA. Areas of low T55 signal, for example near the nuclear envelope and in early G1 cells, do not result from low accessibility to probe molecules since centromeres were readily detected here.

Immunofluorescent Staining of snRNP Antigens

The snRNP mAb used, Y12, is categorized as an anti-SM Ab because it selectively precipitates ribonucleoprotein complexes containing U1, U2, U4, U5, and U6 RNAs, all of which are involved in pre-mRNA splicing (Lerner and Steitz, 1979; Lerner et al., 1981; Petterson et al., 1984; Ringertz et al., 1986; Zieve and Sauterer, 1990) for review). Cell were treated with Y12 in PBS, 1% BSA at 37°C for 45 min (staining in 4x SSC also worked well) and detected using a rhodamine-conjugated goat anti-mouse Ab (Cappel Laboratories, Malvern, PA). For simultaneous detection of poly(A) RNA and snRNPs, T55 was hybridized and detected as above except that anti-snRNP Ab was added during the biotin detection step and was subsequently visualized using a secondary Ab.
Table I. Chromosome 17 Centromere Location

<table>
<thead>
<tr>
<th>Position relative to transcript domains</th>
<th>Investigator number 1</th>
<th>Investigator number 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>In plane coincident</td>
<td>&lt;0.01%</td>
<td>0</td>
</tr>
<tr>
<td>In plane bordering</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>In plane completely separate</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>Out of plane</td>
<td>67</td>
<td>67</td>
</tr>
</tbody>
</table>

Analyses were done on several hundred cells, independently, by the two investigators. For details see Fig. 5 and "Microscopy" in Materials and Methods.

Results

The approach reported here is based on previous methodological studies which identified and optimized conditions for the preservation and detection of DNA and RNA by in situ hybridization (Lawrence and Singer, 1985; Lawrence et al., 1989; reviewed in Lawrence, 1990). Our goal was to provide an accurate overview of the relative distributions of several different nuclear constituents in intact cells, therefore we chose to use standard two-dimensional fluorescence microscopy which made it possible to base conclusions on the analyses of thousands of cells in dozens of experiments. All photographs presented are unprocessed images directly as they appear through the microscope. Computer-assisted reconstructions providing more detailed three-dimensional information based on analysis of a few cells will be presented elsewhere (Carter, K. C., F. Fay, and J. B. Lawrence, manuscript in preparation).

Poly(A) RNA, Centromeric DNA Double Label

Poly(A) RNA was hybridized in paraformaldehyde-fixed cells and detected with avidin as described above. Cells were then re-fixed for 10 min in 4% paraformaldehyde and digoxigenin-labeled centromeric DNA probe was hybridized following denaturation of cellular DNA in 70% formamide 2X SSC as described elsewhere (Johnson et al., 1991b).

Poly(A) RNA, Replicating DNA Double Label

To label replicating DNA in all stages of S-phase, cells in non-synchronous cultures were treated with 25 μg/ml bromodeoxyuridine (BrdU) for 15 min before fixation. Poly(A) RNA was labeled and cells were re-fixed as described in the previous section. Cells were then treated for 10 min in 4N HCl before detection of BrdU with a fluorochrome-conjugated anti-BrdU antibody (Boehringer Mannheim Biochemicals, Indianapolis, IN).

Figure 1. Distribution of Poly(A) RNA within various cell types. (A) Typical nucleus of a human diploid fibroblast (HDF) hybridized with biotinated T55 and detected with fluorescein-conjugated avidin. Note discrete, brightly staining regions (transcript domains) as well as the dim signal throughout the nucleus. Also note the area of low signal just inside the nuclear envelope (arrows). This region is clearly inside the nucleus as shown by counter staining with DNA dyes (see Fig. 3 A). (B) Low magnification view of poly(A) RNA in HDFs. (C) HDFs hybridized as in (A), but substituting A55 for T55 resulting in no observable signal. (Inset) Location of nuclei as shown by DAPI staining. (D–F) Poly(A) RNA distribution in human intestinal smooth muscle cells (D); Mink lung epithelial cells (E; note telophase cell at lower left); and sea urchin coelomocytes (F).
in vivo distribution. For example, discrete regions of T₃₅ signal occurred using various fixation conditions (see Fig. 3 A), but hybridization using a control oligo-dA₅₅ probe resulted in no signal (Fig. 1 C). Additional control experiments are described in Materials and Methods. In repeated experiments we consistently observed that total nuclear poly(A) RNA was not uniformly or randomly distributed, but was concentrated primarily in a relatively small number of distinct sites. Additionally there was often an area of high poly(A) RNA concentration just outside the nucleus, as well as signal more diffusely distributed throughout the cytoplasm to be described in detail elsewhere (Taneja and Singer, manuscript in preparation).

There were approximately 10 to 20 distinctly bordered signal clusters in most nuclei and each cluster ranged from less than one micron to a few microns in diameter. Relative to the signal intensity from single-copy sequences of known size, the dimensions of these regions and the intensity of the signal indicate a minimum of many thousands of molecules per cluster, as confirmed by quantitative microfluorimetry (Carter et al., manuscript in preparation). Most clusters were roughly spherical and did not appear to interconnect with other poly(A) RNA regions on any focal plane; in a few cells there were longer multi-lobed regions. In many cells poly(A) RNA regions seemed to ring nucleoli, but were not seen inside nucleoli. Outside the concentrated poly(A) RNA regions, or transcript domains, there was also faint signal throughout the nucleus, except in the nucleolus (Figs. 1 A and 2), and a region of markedly low signal just inside the nuclear envelope ~1 μm wide (Fig. 1 A and 3 A).

To ask whether nuclear transcript domains occur in other species and cell types, we visualized poly(A) RNA in 26 cell lines and primary cultures from human, rat, mouse, chicken, mink, and sea urchin (Fig. 1, D–F). In each case poly(A) RNA was concentrated in distinct nuclear regions. Furthermore, the encircling of nucleoli with transcript domains was frequently observed and quantitative analysis indicated nucleolar association in HDFs was significantly greater than would be predicted by random distribution of transcript domains (Fig. 2).

Transcript Domains Coincide with Clusters of snRNP Antigens

Messenger RNA is formed through a complex series of steps which includes transcription, 5' end capping, 3' end cleavage, addition of a 3' poly(A) tail, removal of introns, and transport of the mature mRNA to the cytoplasm. It is not known whether any of these steps are physically compartmentalized within the nucleus. There is evidence that transcription and polyadenylation are closely linked (see Discussion), and we reasoned that areas of high poly(A) RNA concentration might be sites of either pre-mRNA transcription, one or more processing steps, a rate limiting step in transport, or...
all of the above. Hence, experiments were undertaken to address whether any relationship could be discerned between the distribution of poly(A) RNA and snRNP antigens involved in splicing.

Double-label experiments, using an antibody specific to snRNPs of the pre-mRNA splicing class, revealed a strong coincidence between poly(A) RNA regions and snRNP antigen clusters (Fig. 3, C and D). This was repeated several times and analyzed using a dual-wavelength filter set which makes possible highly precise comparisons of different wavelength fluorescence distributions. With the exception of recently divided early G1 cells (see below), experiments which gave high detection efficiency for both poly(A) RNA and snRNP antigens consistently resulted in virtually complete overlap of the nuclear immunofluorescence signals. The co-localization of poly(A) RNA and snRNP antigens indicates that transcript domains are not solely transcription or transport sites and that snRNP protein clusters are not simply areas of snRNP assembly or storage. This co-localization supports the interpretation that these regions are sites of pre-mRNA processing.

**Organization of Poly(A) RNA with Respect to the Underlying Genome**

Regions of markedly concentrated poly(A) RNA may form to facilitate the processing and/or transport of RNA in a manner independent of the underlying chromatin. Alternatively, a greater degree of functional organization would be implicated if the RNA was specifically positioned with respect to DNA. Hence, to understand the organizational underpinning of the nucleus it is important to address whether any relationship could be discerned between the distribution of poly(A) RNA and snRNP antigens involved in splicing.

Double-label experiments, using an antibody specific to snRNPs of the pre-mRNA splicing class, revealed a strong coincidence between poly(A) RNA regions and snRNP antigen clusters (Fig. 3, C and D). This was repeated several times and analyzed using a dual-wavelength filter set which makes possible highly precise comparisons of different wavelength fluorescence distributions. With the exception of recently divided early G1 cells (see below), experiments which gave high detection efficiency for both poly(A) RNA and snRNP antigens consistently resulted in virtually complete overlap of the nuclear immunofluorescence signals. The co-localization of poly(A) RNA and snRNP antigens indicates that transcript domains are not solely transcription or transport sites and that snRNP protein clusters are not simply areas of snRNP assembly or storage. This co-localization supports the interpretation that these regions are sites of pre-mRNA processing.

**Figure 4. Detection of low density DNA areas corresponding to poly(A) transcript domains.** A single HDF nucleus is shown, comparing the distributions of propidium iodide (A), and DAPI (B), with poly(A) RNA (C). Arrows are for orientation and to denote typical areas of low DNA which correspond to transcript domains. (D) Nucleus of an unextracted, unfixed HDF grown in the presence of propidium iodide for 30 min. Similar distributions were seen in cells extracted with triton but not fixed before staining.
Figure 5. Demonstration of chromosome 17 centromeric DNA above and below poly(A) transcript domains in a single HDF nucleus. Using a low depth-of-field objective (see Materials and Methods) and standard photography, three focal planes are shown corresponding to the top (A), middle (B), and bottom (C) region of a nucleus. Signal for poly(A) RNA (left) and centromeric DNA (right) are shown. Arrows are for orientation. Note that one centromere is above regions of poly(A) RNA and the other is below, while out-of-focus light from both centromeres can be seen in the central plane.

correspond to transcriptionally active and inactive chromatin and localize to light and dark G-bands in metaphase chromosomes, respectively (reviewed in Bickmore and Sumner, 1989; Herbomel, 1990); hence, it becomes compelling to consider whether this non-homogeneous distribution of functional DNA classes, clearly visible at metaphase, is related to the clustered distribution of gene transcripts in interphase nuclei. Also, in light of current hypotheses which call for significant movement of DNA during replication (reviewed in Laskey et al., 1989), it was of interest to determine whether transcript domains remain intact throughout S-phase.

A two-color fluorescence protocol was developed which allowed simultaneous detection of poly(A) RNA and replicating DNA. Non-synchronous cultures were labeled for a short period (15 min) with BrdU, and many cells were analyzed so that the distribution of transcript domains could be compared with a full spectrum of different S-phase replication patterns (Fig. 6). The first thing these experiments demonstrated was that there is essentially no change in the general pattern of transcript domains during S-phase. An examination of hundreds of cells in multiple experiments showed that none of several replication patterns observed coincided with the pattern of poly(A) RNA regions. Hence, the transcript domains do not correspond in any obvious way to subsets of synchronously replicating DNA which become organized as bands on metaphase chromosomes.

While the overall patterns of poly(A) RNA and replicating DNA were clearly not the same, another question concerns whether there was any relationship or overlap between them. Several different patterns of replicating DNA were seen, and in many cells it was possible to identify patterns similar to those previously described for late- or early-replicating DNA (Nakayasu and Berezney, 1989). In most cells containing the clustered pattern characteristic of late-replicating DNA, poly(A) RNA regions and replicating DNA were consistently discernible as non-overlapping (Fig. 6 C). In contrast, in nuclei with the more finely distributed punctate pattern characteristic of early S-phase, during which most active genes replicate, some replicating DNA frequently appeared to overlap poly(A) RNA regions in the same focal plane (Fig. 6 A). This suggests that these regions may contain or be closely associated with subsets of early replicating DNA, however, further details will require in-depth three-dimensional analysis. In some cells, replicating DNA occupied the peripheral region near the nuclear envelope where poly(A) RNA concentration was markedly low, producing a striking ring of fluorescence (Fig. 3, G and H).

These data indicate that there is not a simple interphase coincidence between transcript domains and the functional subsets of DNA seen as metaphase bands. However, they do

Figure 6. Simultaneous visualization of poly(A) RNA (left) and replicating DNA (right). Three of several replicating DNA patterns seen are shown (see text for details). Many cells had replicating DNA patterns which seemed to be typical of those previously described for early (A) or late (C) S-phase. Careful analysis with a low depth-of-field objective indicated that patterns like those in (B) and (C) had little or no overlap with transcript domains.
suggest a spatial relationship. Furthermore, if splicing does occur in transcript domains, these results provide a clear demonstration of the differential nuclear compartmentalization of two distinct physiological processes, that of DNA replication and RNA processing.

**Nascent Poly(A) Transcripts May Be Involved in the Reclustering of snRNPs following Mitosis**

The existence of this distinct nuclear compartment, analogous to the nucleolus in that it may be responsible for processing of a major class of RNA, raises questions about the fundamental mechanisms which establish and maintain functional compartmentalization within the nucleus. Recently divided cells were readily apparent in our experiments (Fig. 1E, and 3B), hence, to study the process of assembling transcript domains during early G, we analyzed several hundred mitotic and postmitotic cells in preparations stained simultaneously for poly(A) RNA and snRNP antigens (Fig. 7). At metaphase, anaphase, and telophase, chromosomes were essentially devoid of both poly(A) RNA and snRNP although signal for each was seen throughout the cytoplasm. In telophase cells and newly divided daughter cells poly(A) RNA signal was often heavily concentrated just outside the nuclear envelope, but markedly absent inside the nucleus (Figs. 3B, and 7C). This indicates that even though poly(A) RNA concentration remains high during mitosis and surrounds mitotic chromosomes, this RNA is efficiently excluded from the nucleus as the nuclear envelope reforms.

In sharp contrast to other interphase cells, the early G1 nuclear poly(A) RNA pattern was very different than that of snRNPs. In what appeared to be the earliest G1 cells, snRNP antigens were seen in various stages of re-entry into the nucleus while nuclear poly(A) RNA remained undetectable. In these cells snRNP antigens had often re-entered the nucleus, but were distributed relatively uniformly or in a fine punctate pattern (Fig. 7C). In apparently later daughter cells a dim poly(A) RNA signal was present in regions where snRNP antigens had begun to cluster (Fig. 7D). In repeated experiments, poly(A) RNA was not seen in early G1 nuclei in the absence of snRNP signal and snRNP proteins were not discretely clustered in the absence of poly(A) RNA signal. In addition, the discrete low density DNA areas, which correspond to transcript domains, appeared only in early G1 cells in which poly(A) RNA was detectable. These data suggest that the clustering of snRNP antigens into discrete regions after mitosis occurs only after the appearance of poly(A) RNA, presumably following the establishment of transcription by RNA polymerase II (pol II).

**Discussion**

This work describes several previously unknown aspects of the functional organization of the nucleus. In cells ranging from sea urchin to human, nuclear poly(A) RNA was concentrated in several discrete transcript domains which often surrounded nucleoli. These areas appeared to be internally located, had defined borders, and corresponded to areas of very low DNA concentration. There seemed to be complete overlap in these regions between poly(A) RNA and snRNP antigens, providing the best evidence to date that these are sites of pre-mRNA processing. Placement of these areas with respect to total DNA, replicating DNA, and centromeric DNA strongly indicates that these putative processing areas are specifically positioned with respect to underlying chromatin, which may have profound implications for higher-level nuclear organization. Transcript domains were clearly separate from areas of late replicating DNA indicating a distinct physical partitioning of two separate nuclear functions during late S-phase. Finally, our data indicate that the clustering of snRNPs into discrete nuclear domains in early G1 and the appearance of corresponding low density DNA regions occur only after the reappearance of poly(A) RNA. This raises the interesting possibility that nascent pol II transcripts play a significant organizational role in the formation of snRNP clusters, similar to the postulated role of pol I transcripts in the assembly and maintenance of nucleoli (reviewed in Scheer and Benevente, 1990).

**The Relationship of Transcript Domains to pre-mRNA Processing**

While snRNP antigens have been described by several laboratories to have various degrees of clustering (see Introduction), the subnuclear distribution of total poly(A) RNA, the major substrate for snRNP processing, has not been previously investigated. Our results support the functional significance of clustered snRNP protein distributions (Fakan et al., 1984; Nyman et al., 1986; Ringertz et al., 1986; Spector, 1990) and, moreover, provide the first evidence for these as active processing centers rather than assembly and storage sites. Since the completion of the work presented here, the validity and role in processing of previously reported clustered snRNP distributions has been called into question by Carmo-Fonseca et al. (1991a,b), who present results interpreted to indicate that essentially all of the extremely abundant snRNAs are localized together in just one to four small nuclear foci. The interpretation of these few foci as the nuclear processing centers is difficult to reconcile with our results which co-localize pre-mRNA and snRNPs.
snRNP antigens to 10–20 widely distributed domains of relatively equal intensity, or with the observation that the snRNA cap structures localize to snRNP antigen clusters (Reuter et al., 1984). The potential for these areas in RNA processing is also supported by recent studies showing that microinjected β-globin pre-mRNA co-localizes with snRNP clusters (Wang et al., 1991).

Our work also supports the validity of clustered snRNP patterns by defining a physical compartment in the nucleus which was observed using three separate approaches: (a) in situ hybridization to poly(A) RNA; (b) immunofluorescence to snRNP particles; and (c) visualization of total DNA with fluorescent dyes. This, along with the fact that these regions were apparent in unfixed cells stained for DNA, greatly increases the confidence with which it can be concluded that these are genuine structural and functional nuclear compartments. Our data are consistent with numerous EM studies indicating interior nuclear regions of low DNA density (reviewed in Fakan, 1978; Fakan and Puvion, 1980; Fawcett, 1981) in which snRNP antibodies localize (Fakan et al., 1984), but do not necessarily support the conclusion that these regions contain no DNA (Spector, 1990). It is extremely difficult to distinguish between the presence of no DNA and little DNA in these regions using EM or by advanced fluorescence imaging using DNA-specific dyes (Carter et al., manuscript in preparation), hence a conclusive answer to this question awaits more extensive analyses.

**Does Transcription Occur in These Regions?**

There are at least three reasonable models that would explain why the vast majority of nuclear poly(A) RNA resides in these defined transcript domains. First, this RNA might be transcribed elsewhere and be transported to these regions to be polyadenylated and spliced. Similarly, this RNA might be both transcribed and polyadenylated elsewhere and then transported to these sites for further processing which would be rate limiting. Alternatively, transcription, polyadenylation, and splicing may all occur within transcript domains, in which case any one of these processes could be rate limiting and cause a build-up of transcripts.

Several lines of evidence presented here are consistent with the possibility that poly(A) RNA regions are transcription sites. These include: (a) The distribution of poly(A) RNA is not random with respect to total DNA; (b) these discrete regions contain very little DNA, which one might expect in areas of active transcription since much evidence indicates that active chromatin is decondensed (Weisbrod, 1982; Lewin, 1990); (c) centromeric and late replicating DNA, which are transcriptionally inactive, are completely excluded from these regions; and (d) early-replicating DNA, which contains most active genes, was not preferentially excluded and seemed to partially overlap poly(A) RNA regions. It is tempting to visualize these regions as filled with loops of decondensed transcriptionally active DNA analogous to structures seen on amphibian oocyte lambrush and drosophila polytene chromosomes. However, poly(A) transcript domains would reflect a significantly different level of organizational complexity; with each poly(A) RNA domain reflecting transcriptional activity from hundreds or thousands of individual genes on different chromosomes rather than the activity of a single atypically large or amplified transcription unit. However, short of localizing specific active genes directly within these regions, it cannot be ruled out that there is nonspecific exclusion of DNA in this region imposed by the concentration of transcripts and splicing machinery.

The fact that snRNP antigens are located within transcript domains may provide indirect evidence that pol II transcription occurs at these sites: Transcription by pol II is necessary for polyadenylation and splicing in vivo (Smale and Tjian, 1985; Lopata et al., 1986; Sisodia et al., 1987) and EM studies have shown RNP particles and apparent splicing on nascent pol II transcripts (Beyer et al., 1981; Osheim et al., 1985; Beyer and Osheim, 1988) and snRNP antigens within spread chromatin (Fakan et al., 1986). Likewise, a functional polyadenylation signal is necessary for the termination of pol II transcription, indicating a tight linkage between 3' end processing and transcription (Falk-Pederson et al., 1985; Whitelaw and Proudfoot, 1986; Logan et al., 1987; Connelly and Manly, 1988), and at least two studies indicate that non-transcribed promoter region sequences can affect mRNA processing and transport (de la Pena and Zasloff, 1987; Neuberger and Williams, 1988). However, because both polyadenylation and splicing occur on pre-mRNAs added exogenously to nuclear extracts, and certain full-length primary transcripts are seen by Northern analysis, the possibility remains that the progression from transcription to polyadenylation and splicing takes place in a linked manner but that the physical compartmentalization, in some cases, may be separate.

**Further Implications for Nuclear Structure and Function**

If transcript domains do represent transcription sites, this would indicate that active chromatin is distributed in clusters throughout the interior portion of the nucleus. Direct and conclusive evidence for the general location of active genes at interphase is lacking. Several laboratories using EM autoradiography have observed nascent RNA at sites throughout the nuclear interior, in some cases proximal to structures of unknown function seen in EM micrographs (reviewed in Fakan and Puvion, 1980). In contrast, others have shown DNase I-sensitive DNA, presumed to represent transcriptionally active chromatin, primarily near the nuclear envelope in some cell types (Hutchinson and Weintraub, 1985; Kryostek and Puck, 1990). However, many studies indicate that the bulk of DNA, including centromeres and the inactive X chromosome, occurs in heterochromatic regions at the nuclear periphery (Fawcett, 1981; Ford, 1973). In this context an earlier study noted that poly(A) RNA was distributed throughout the nucleus and not just at the periphery, although subnuclear details were not investigated (Bauman et al., 1990).

Consistent with a more interior positioning of active chromatin, our previous work directly localized the Epstein-Barr Virus genome to the inner 50% of the nuclear volume in lymphoma cells where it is abundantly transcribed (Lawrence et al., 1988, 1989). Similar positioning was seen for the Human Immunodeficiency Virus (HIV) genome in productively infected cells (Lawrence et al., 1990) and preliminary studies suggest an interior position for some endogenous active pol II genes (Xing, Johnson, and Lawrence, manuscript in preparation). Tracks of EBV nuclear RNA extend from the internally localized gene into the outer 50%
of nuclear volume, often appearing to contact the edge of the nucleus (Lawrence et al., 1989) and are completely preserved both spatially and quantitatively during nuclear matrix preparations (Xing and Lawrence, 1991). Thus, while internal localization of poly(A) RNA in transcript domains is difficult to reconcile with often cited views of nuclear organization which propose localization of active chromatin at the nuclear periphery, our data are not necessarily inconsistent with proposals that the nuclear lamina or pores might function in nuclear organization (for example, Blobel, 1985). The potential compartmentalization of various functions seen here and in previous work from our lab and others' concerning the association of RNA (Xing and Lawrence, 1990; reviewed in Fey et al., 1991) and replicating DNA (Nakayasu and Berezney, 1989; reviewed in Nelson et al., 1986) with the nuclear matrix make it reasonable to suggest that the nuclear matrix may play a role in this physical partitioning of various nuclear functions.

The observation that poly(A) RNA is often preferentially concentrated around the nucleolus raises questions as to whether this structure or the region immediately surrounding it plays a role in mRNA transport and/or processing. Interestingly the HIV rev protein, a predominantly nucleolar protein, is involved in transport of HIV mRNAs from the nucleus (Cullen et al., 1988; Cochrane et al., 1989; Lawrence et al., 1991). It is possible therefore that a direct association exists between mRNA transport and nucleolar function. For example, it is conceivable that ribosomal subunits or other nucleolar constituents are physically associated with mRNAs during transport.

In conclusion, we have provided evidence for functionally significant physical partitioning of RNA and DNA within the nucleus, and have described a compartment which is likely to play a major role in pre-mRNA processing and, possibly, transcription. Furthermore, the relative ease with which this major intra-nuclear compartment can be visualized by any of the techniques used, will provide a landmark for studying the precise relative placement of other important nuclear constituents such as active genes.

We would particularly like to acknowledge Robert Singer for his support and input. We thank John Coleman, Ted Fey, and Rob Singer for critical reading of the manuscript. We thank Ted Fey for HDF cells, Thoru Pederson for Y12 Ab, and Kip Sluder for sea urchin cells. We are also grateful to Chris Dunshee, Mike Gerdes and Marie Picard-Craig for assistance in the preparation of the figures and Cindy Beaudeyr for secretarial assistance.

This work was supported by an R.C.D.A. to J.B.L. and grants from the National Center for the Human Genome (HG 00251) and the Muscular Dystrophy Association. K.C.C. was a Jeanne B. Kempner and Muscular Dystrophy Association in post-doctoral fellow during this work.

Received for publication 13 May 1991 and in revised form 30 August 1991.

References


Herbomel, P. 1990. From gene to chromosome: Organization levels defined by the interplay of transcription and replication in vertebrates. The New Biologist. 2:937-945.


Hutchinson, N. and H. Weinstein. 1985. Localization of DNase I-sensitive se-