DNA and RNA Within the Nucleus: How Much Sequence-Specific Spatial Organization?

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Abstract   Spatial organization of various nuclear components is often proposed as a means by which nuclei more efficiently carry out their various tasks. Such functional compartmentalization may involve a sequence-specific packaging and placement of DNA and RNA. Here we review recent insights, allowed primarily by advances in fluorescent in situ hybridization methodology, into the organization of nucleic acids within individual nuclei.

Key words: chromatin, genome organization, in situ hybridization, nucleus, RNA processing, RNA transport

It has often been noted that there is a strong rationale for functional spatial organization in the nucleus [see for example Comings, 1968, 1980]. This rationale is in part based on the numerous complex tasks the nucleus must perform, the distinct biochemical machineries necessary to accomplish each of these tasks, and the extreme density of the nucleoplasm with its theoretical limitations on diffusion [for a recent review see Jackson, 1991]. A major nuclear function is packaging the enormous length of genomic DNA in such a way that it is accessible for replication, selective transcription, and accurate division during mitosis. Likewise, RNA transcripts from individual genes, which can be several microns long, must be specifically and precisely processed and selectively transported to the cytoplasm. The rapid kinetics of RNA transport from the nucleus suggest that this is not accomplished by diffusion but rather requires some vectorial, energy-dependent process. With the exception of the nucleolus, organizational details of the nucleus are poorly understood; but it is clear that any functional compartmentalization will likely involve directed sequence-specific positioning of DNA and RNA. Although perhaps not widely appreciated, the precedence for this is well established by the nucleolus, in which rRNA genes from five different human chromosome pairs are brought together during interphase for the common purpose of ribosomal RNA transcription and subunit assembly.

The spatial organization of specific nucleic acids within the nucleus may also be important during development. For many years cytological and histological studies have documented cell- and tissue-type specific changes in nuclear architecture; however, the functional significance of why nuclear morphology often varies radically and reproducibly with cell-type is largely unexplored. Considering that the process of development involves a series of determination steps which represent a progressive decrease in the developmental competence of individual cells, it becomes plausible to suggest that nuclei may undergo organizational changes which reduce the accessibility of specific gene sets to factors which stimulate transcription. While great strides have been made in describing the interaction of transcription factors with individual genes, understanding the highly orchestrated control over total genome expression required during the development of higher eukaryotes is not currently within grasp. It is possible that the spatial organization of the genome may play a substantial role. A precedent for such functionally significant organizational changes of specific genes is again hinted at by nucleoli, which vary in number and location between functionally distinct cell-types.

Recent advances in high resolution detection of specific DNA and RNA sequences by fluorescent in situ hybridization have provided new insights into the organization of nucleic acids at interphase and metaphase and provide a powerful new approach for studying the placement of

specific sequences within the nucleus [reviewed in Lawrence, 1990]. Here we review recent advances in understanding the functional partitioning of the nucleus concentrating on the placement of individual genes and their nuclear transcripts.

HIGHER-LEVEL CHROMATIN PACKAGING: INTERPHASE AND METAPHASE

During the past two decades there has been a great advance in our understanding of chromatin structure at its two lowest levels of packaging: the nucleosome and the 30 nm fiber [Weisbrod, 1982]. However, beyond this several more forms of packaging must exist to condense 2 meters of linear human genome approximately 10,000 times to fit within a 5 micron nuclear diameter. An important observation which indicates a high degree of organization at a gross level is the reproducible pattern of dark and light staining bands on metaphase chromosomes [reviewed in Bickmore and Sumner, 1989]. Only in very recent years have we begun to understand the implications of these banding patterns in terms of functional organization. The Giemsa light bands correspond to chromatin that is gene-rich, early replicating, DNAse sensitive, and enriched in specific classes of repetitive sequences. This apparent partitioning of functional classes of DNA on chromosomes is likely to have some corollary within the interphase nucleus. In fact it is well documented that active genes are generally early replicating [see Herbomel, 1990, and references therein], and that early and late replicating DNA show characteristic and distinct spatial distributions at interphase [Nakayasu and Berezney, 1989].

The actual mechanics of how chromatin is packaged to form a functionally organized, banded chromosome remain unknown. There is evidence that the 30 nm chromatin fiber is packaged into loops at both interphase and metaphase which vary in size from tens to hundreds of kb [see Gasser and Laemmli, 1987; Jackson, 1991, and references therein]. While several groups have postulated how these loops are packaged [for example: Nelson et al., 1986; Jackson, 1986; Gasser and Laemmli, 1987; Manuelidis and Chen, 1990] hard evidence supporting any one model is lacking. The continuing development and application of high-resolution, high-sensitiv-

ity in situ hybridization techniques promises to make a major contribution to this area.

Recent measurements of physical distances within the dystrophin gene contributed several new observations and insights bearing on models of higher-level chromatin packaging (Fig. 1F) [Lawrence et al., 1990]. For instance, dystrophin gene sequences up to 1 Mb apart can be resolved as distinct signals across the width of the chromatid axis, but not along its length. This provides direct evidence for the path followed by chromatin as it folds into chromosomes. The packaging ratio at 100 kb for the dystrophin gene was 1:73 in interphase cells where it is inactive. It will be pertinent to understanding higher-level packaging to determine whether this changes when the gene is active and whether this ratio is common to other regions of the genome. For three published regions of the human genome in this size range the packaging ratio is similar and only slightly higher than the 1:40–1:50 ratio predicted for the 30 nm fiber [Lawrence et al., 1988; Lawrence et al., 1990; Trask et al., 1990]. At present, conclusions from such measurements must be made cautiously since it is not known how fixation affects higher level chromatin structure; but it is encouraging that very similar physical separations have been observed for paraformaldehyde fixed whole cells as for nuclei of cytogenic preparations [Lawrence et al., 1990].

DNA ORGANIZATION AT INTERPHASE

As long as a hundred years ago, microscopists proposed that chromosomes occupy distinct territories in the nucleus and are aligned in a polarized configuration with centromeres to one end of the nucleus and telomeres to the other [see Rabl, 1885; reviewed in Comings, 1980]. More recent studies have shown that the Rabl orientation does exist in some but not all cell types [see for example Mathog et al., 1984; Foe and Alberts, 1985]. Experiments using total chromosome hybridization and other approaches have shown that individual chromosomes can occupy discrete, relatively compact domains within the interphase nucleus [Cremer et al., 1982; Pinkel et al., 1988; Lichter et al., 1988; Manuelidis and Borden, 1988]. In situ hybridization to large blocks of repeated sequences and immunolocalization with centromere specific antibodies indicate that specific centromeres tend to occupy preferential positions, which in some cases are cell-type or cell cycle stage specific
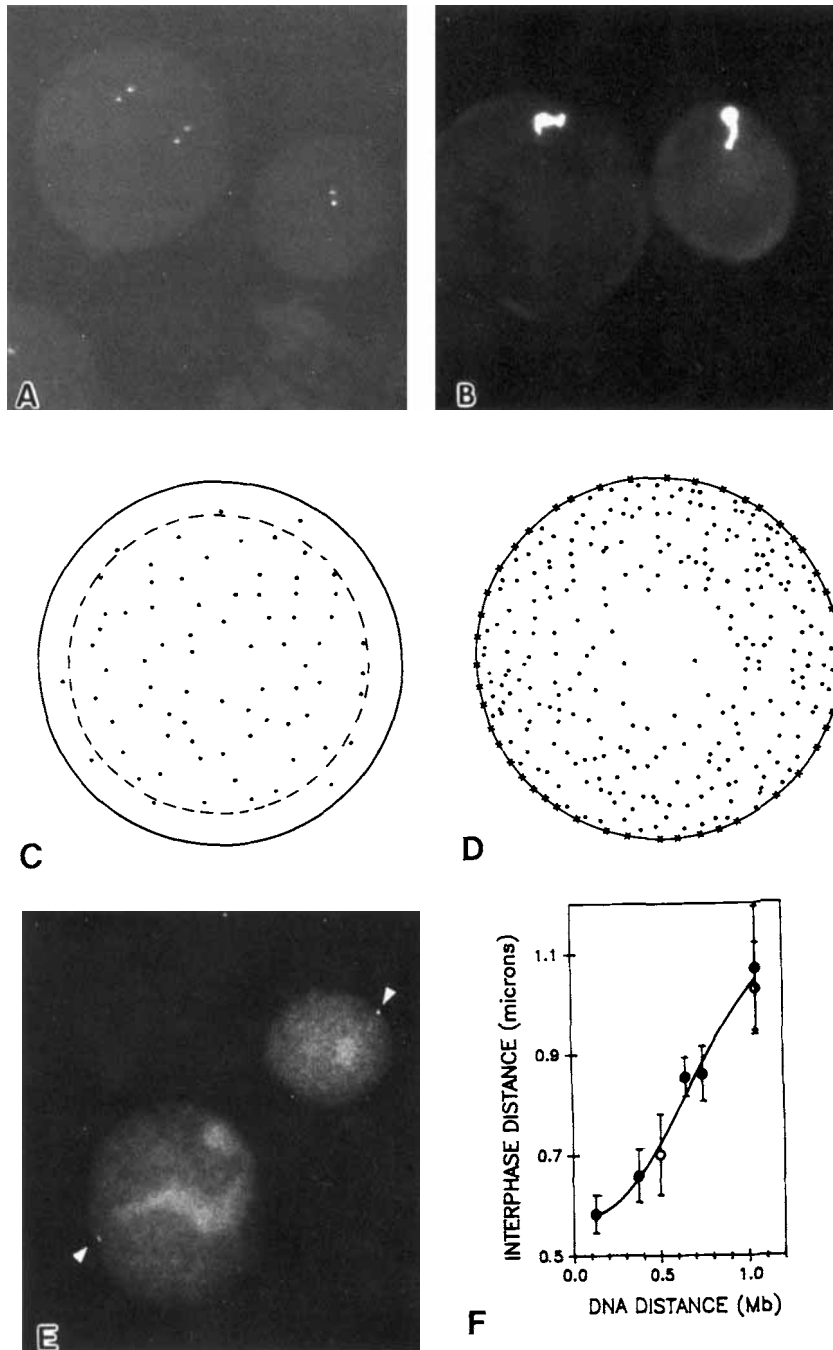
Fig. 1. (A–D) Interphase position of integrated EBV DNA and expressed RNA in nuclei of a lymphoma cell line, as determined by fluorescence in situ hybridization (Lawrence et al., 1988, 1989). (A) Two closely-spaced EBV genomes integrated on a single chromosome a few hundred kb apart. Nucleus at right is haploid and in G_1 while nucleus at left is either tetraploid or in G_2. This demonstrates the ability of this approach to resolve closely linked sequences on the same chromosome and to determine the relative positions of either homologous or replicated sequences. (B) Tracks of EBV RNA in isolated nuclei from cells containing a single integrated viral genome. RNA tracks within two nuclei are shown; DNA counter-stain is not pictured. (C) Position of integrated EBV genome in freely rotated nuclei of intact cells in which the viral DNA is highly tran-

scribed. DNA signals were essentially confined to the inner 50% of nuclear volume (corresponding to the internal 80% of the radius). (D) Position of the most distal point of the EBV RNA tracks in freely rotated nuclei of intact cells. (E) Peripheral position of the dystrophin gene in peripheral blood lymphocytes—a cell type in which this gene is not expressed. (F) Average interphase distance between sequences within the dystrophin gene (±SEM) in peripheral blood lymphocytes (circles) and primary G_1-arrested fibroblasts (triangle). Note the strong correlation between DNA distance and interphase distance within the range shown. (Reproduced from Lawrence et al. with permission of Science 249:928-932, copyright 1990 by the AAAS).

[reviewed in Hadlaczky et al., 1986; Manuelidis, 1990; Haaf and Schmid, 1991].

The functional organization of active genes within the nucleus remains largely unknown. Indirect evidence based on DNAse I sensitivity suggests a general location of active chromatin around the nuclear periphery in some cells [Hutchison and Weintraub, 1985; Krystosek and Puck, 1990]; however, studies from a number of laboratories show heterochromatin at the nuclear periphery [see Comings, 1980] and E.M. autoradiography shows nascent radiolabelled RNA at sites throughout the nuclear interior [reviewed in Fakan and Puvion, 1980]. The nucleolus, which represents the singular known example of coordinated placement of specific genes, is known primarily because its extreme density renders it immediately obvious by simple phase microscopy. An approach to the fundamental question of whether other similar but less readily identifiable functional compartments of gene sets exist has awaited the recent development of techniques which allow high-resolution visualization of specific sequences.

While the interphase position of specific genes has not been widely studied, some information is available. For an integrated Epstein-Barr Virus (EBV) genome in lymphocytes, analysis of large numbers of freely rotated nuclei demonstrated that the active EBV genome was non-randomly positioned and restricted to an inner nuclear sphere representing approximately 50% of the nuclear volume (Fig. 1A,C) [Lawrence et al., 1988]. Thus, at least in some cases, active pol II genes are positioned interiorly, as opposed to the region next to the nuclear envelope. Because nuclei had been randomly rotated, the analysis of signal position provides localization with respect to the Z-planes, but does not eliminate the possibility of an even more specific placement with regard to X-Y coordinates. We have also seen similar non-random placement of endogenous genes. For example, neuoncogene sequences are consistently internally localized whereas inactive dystrophin sequences are very close to the nuclear envelope even in male cells in which the X chromosome is not inactivated (Fig. 1E) [Lawrence et al., 1990]. Analysis of several genes in the active and inactive states will be necessary to determine how generalizable these observations are.

Visualization of specific sequences at interphase also allows one to address questions about somatic pairing of homologous sequences. It had been previously shown that homologous centromeres do not generally lie closely juxtaposed [see Haaf and Schmid, 1991]. However, the question of whether genes in their active or inactive states are somatically paired, as they are in Drosophilia polytene chromosomes, has only been recently addressed. We have reported for four different individual genes that the homologous sequences are not somatically paired within lymphocytes or fibroblasts [Lawrence et al., 1990], and have since observed this for numerous other sequences including transcriptionally active genes [Xing et al., in preparation]. In contrast to homologous sequences, replicated sequences usually lie very close to each other but usually far enough apart to be resolved as two separate but closely spaced spots.

SEQUENCE-SPECIFIC ORGANIZATION OF RNAS

The nucleus must not only accurately transcribe, process, and transport RNA through a nuclear interior densely packed with chromatin; it must do so in a way which is highly selective. Several major classes of RNA, including tRNA, rRNA, snRNA, and mRNA, are metabolized by distinct sets of enzymes. Moreover, the nucleus somehow discriminates between processed and unprocessed RNA, and exports only functionally appropriate molecules such as mature mRNA. Remarkably, in the case of snRNAs, export is followed by cytoplasmic processing after which the RNA returns to the nucleus [see Zieve and Sauterer, 1990].

Very little is known about the nuclear distribution of specific sets of RNA. Although rRNA is easily visualized within the nucleolus by in situ hybridization or by general RNA dyes such as propidium iodide and acridine orange, the distribution of this RNA during nucleocytoplasmic transport as part of ribosomal subunits remains essentially unknown. One recent report indicates that snRNAs are concentrated in approximately 4 small nuclear foci in many cell-types, but the functional significance of this awaits further investigation [Carmo-Fonseca et al., 1991].

Several observations from our lab provide more detailed insights into the functional organization of pre-mRNAs. The first of these was the visualization of transcripts from an integrated virus in highly localized, elongated "tracks" which often traversed over one-fourth the nuclear diameter (Fig. 1B,D) [Lawrence et
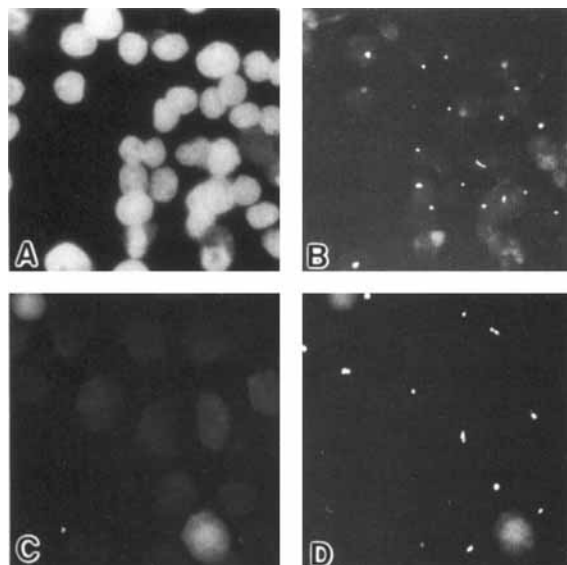
Fig. 2. Retention of EBV RNA tracks following biochemical fractionation which removes approximately 90% of DNA, protein and phospholipid. **(A and B):** Nuclei from intact, unextracted lymphoma cells showing DAPI staining of total DNA (A), and detection of EBV RNA tracks (B). **(C and D):** same as (A) and (B) but following nuclear matrix fractionation. Note dramatic loss of DNA (C), but retention of EBV tracks (D). (Reproduced with permission of Cell Press 52:51–61, Xing and Lawrence, 1990).

al., 1989]. These tracks represent an accumulation of hundreds of transcripts which extend well beyond the dimensions of the gene. Hence, they are not equivalent to the "Christmas tree" of nascent transcripts observed at ultrastructural resolution in E.M. spreads [Beyer et al., 1981], but rather most likely represent transcripts at some point along the processing/transport pathway. The existence of RNA in highly defined tracks is consistent with a solid-state model for the nucleoplasm wherein RNA is physically constrained via association with nuclear substructure [reviewed in Nelson et al., 1986; Jackson 1990; Fey et al., 1991]. This is further suggested by the fact that the RNA localization becomes even more dramatic and elongated in the swollen nuclei of cytogenetic preparations, rather than becoming more diffuse, as though the RNA becomes distended along with the nuclear structure.

By coupling nuclear fractionation procedures with in situ hybridization we were able to directly address the possible association of EBV RNA with the underlying substructure termed the nuclear matrix (Fig. 2) [Xing and Lawrence, 1991]. This demonstrated that tracks of viral RNA are completely preserved both quantita-

tively and morphologically throughout fractionation procedures which remove 95% of nuclear protein, phospholipid, and DNA. Since RNA localization was unambiguously maintained in the absence of bulk DNA, this localization is not a consequence of compression due to spatial constraints imposed by the dense chromatin. Because the in situ approach demonstrates that a *specific* RNA is spatially as well as quantitatively maintained in nuclear matrix preparations, these results provide direct visual evidence which strengthens a body of somewhat controversial literature supporting the association of newly synthesized transcripts with the matrix [see Fey et al., 1991, for review]. Interestingly, in cells which carry many episomal viral genomes there are many nuclear tracks of RNA present in each which are also precisely preserved within the matrix [see cover figure, this issue, and Xing and Lawrence, 1991]. While the EBV model system has been excellent for revealing new aspects of nuclear organization and structure, it cannot a priori be considered representative of all gene transcripts. The nuclear abundance and spatial configuration of a given pre-mRNA is likely to depend greatly on the position of the gene, size of the primary transcript, extent of processing, and level of transcription. Our recent studies indicate that many endogenous genes have RNA tracks or foci associated with each homolog, showing varying degrees of similarity to the viral RNA tracks [Xing et al., in preparation].

Very recently in a detailed study of total pre-mRNA distribution using fluorescence hybridization to poly(A) RNA, we found a compartmentalization of pol II transcripts in discrete domains throughout the nucleus [Carter, Taneja, and Lawrence, in press]. These results strongly indicate that RNA metabolism is not uniform throughout the nucleus, but concentrates in defined "hot spots." Examination of these transcript domains with respect to various functionally defined nuclear constituents has revealed strong evidence that these are areas of pre-mRNA processing, and possibly transcription. Hence these regions may reflect a non-homogeneous, non-peripheral distribution of active genes.

FUTURE DIRECTIONS

The collective evidence from several different laboratories using a variety of approaches indi-

cates that there is a large degree of sequence-specific spatial organization of DNA and RNA within the nucleus. We are just beginning, however, to understand how these specific localizations relate to nuclear function. An important avenue of future research will be to determine rigorously what organizational schemes exist for active genes. The emerging story concerning the compartmentalization of major classes of nuclear RNA and processing components will be fundamental to understanding overall functional organization, and ultimately must be investigated with respect to the location of active chromatin. How does the spatial configuration of these components change during development? While it is possible that a complex balance of transcription factors will prove responsible for the progressive and heritable narrowing of epigenetic potential which occurs during development, coordination of total genome function may in part be regulated at the level of structural organization.

If DNA and RNA are spatially organized within the nucleus, then how is this organization established and maintained? Ultimately the organization of nucleic acid sequences must be understood in relation to nuclear structural elements. A powerful visual approach to such questions is provided by coupling in situ hybridization with now well-defined biochemical fractionation techniques. In addition to using this approach to visualize RNA associated with the matrix, most recently we have been able to visualize single-copy DNA sequences directly on specific DNA loops within fractionated nuclei [Gerdes, Carter, and Lawrence, in preparation]. This may provide an experimental approach to directly investigate these loops within single cells. While many of these questions are best addressed at the light microscopic level, electron microscopic in situ hybridization may enhance the power of this approach further for addressing questions which require ultrastructural resolution. The development of different approaches from a number of laboratories promises to make the next decade an exciting one for advancing our understanding of nuclear structure/function relationships.

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