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Nuclear RNAs confined to a reticular compartment between chromosome territories

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Abstract

RNA polymerase II transcripts are confined to nuclear compartments. A detailed analysis of the nuclear topology of RNA from individual genes was performed for transcripts from the marker gene coding for chloramphenicol acetyltransferase, expressed at a high level from the HTLV-1 LTR promoter. The construct was transfected into A293 cells where the RNA was organized as an extensive reticular network. We also studied the RNA distribution from combinations of neighboring HIV and bacterial resistance genes that co-integrated within the genome of COS-7 cells—revealing spherical or track-like accumulations of RNA that were extensively branched. There were many nuclei with distinct but overlapping RNA accumulations. Since the coding genes localized at the overlapping points, the RNAs are synthesized at a common region and diverge. The correlation between the frequency of the separation of the transcripts and the physical distance of the respective genes suggests a subcompartmentalization in the microenvironment of genes on the basis of geometric parameters. Thus, the more distant the genes are on the same chromosome, the more likely they are confined to separated subcompartments of an extensive reticular system. Co-delineation of the RNA transcripts with Cajal bodies and chromosome territories indicated the organization of nuclear RNA transcripts in a reticular interchromosome domain compartment.

Keywords: Nuclear RNA; Chromosome; Reticular compartment

Introduction

There is a high level of organization and compartmentalization within higher eukaryotic nuclei. Nuclear organization and compartmentalization is apparent when factors that are involved in metabolic processes such as replication, transcription, and splicing are visualized: Many of these are distributed throughout the nucleus in discrete accumulations and foci. Most factors involved in the replication machinery are localized in a punctuate pattern at the onset of S-phase in mammalian cells that progresses to a more granular distribution with fewer larger foci late in S-phase [1–4]. Similarly, factors involved in transcription are also distributed throughout the nucleus in an organized manner, in discrete foci or accumulations. Transcription factors, RNA polymerase II, nascent RNA, poly(A+) accumulations, total pre-mRNA, and messenger RNA as well as specific nuclear RNAs have all been shown to be distributed in a distinct fashion at discrete sites [5–11].

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In a number of studies, individual species of RNA have been visualized by fluorescence in situ hybridization (FISH), such as nuclear RNA derived from Epstein–Barr virus, human papilloma virus, cytomegalovirus, fibronectin, or neurotensin gene [5,9,11–14]. The RNA from these highly expressed genes appears as large accumulations of either spherical or track-like morphology. Where the RNA is distributed as a nuclear track, it has been shown that the gene coding for the transcripts is positioned at one end of the track or within the track and is point-like in morphology. Thus, the gene is not elongated along the RNA track but the RNA is generated from one discrete area [11,15]. Radiation of RNA molecules through restricted nuclear space was observed in fixed [14] as well as in living cells, where RNA was visualized by photoactivation of caged fluorochromes that are conjugated to the RNA [16,17].

Both sites of replication and transcription are apparently fixed and remain associated with the structures in nuclei after a nuclear matrix or nucleoskeleton preparation has been performed [18,19]. Replication and transcription patterns appear to be similar but localize to different compartments in the nucleus [20,21]. This is also observed for nonintegrated viral genomes [22,23]. Transcription factors are also distributed within the nucleoplasm as discrete foci and do not always co-localize with accumulations of RNA polymerase II [10]. Thus, factors involved in transcription, including the RNA polymerase II, are organized at sites that are not being actively transcribed, implying that some of the focal accumulations are storage sites. This also seems to be the scenario with components involved in splicing, since they are apparently stored and/or pre-assembled (i) in Cajal bodies harboring newly assembled snRNPs and other spliceosome factors but not SC35 (see Ref. [24]), or (ii) in the large 'speckles', which are also termed SC35 domains, since they contain mature splicing snRNPs and splicing factor SC35, and which correspond to the interchromatin granules detected by electron microscopy (see Ref. [25]). While splicing factors are recruited from speckles to areas of active transcription, RNA processing is observed for some genes to be associated with SC35 domains [26,27]. However, the specific organization of the recruitment of the transcription machinery remains elusive, and it is still not fully understood whether splicing occurs cotranscriptionally for all transcripts.

Much of the RNA produced in mammalian nuclei is exported from the nucleus to be translated into protein in the cytoplasm. Recently, it became apparent that splicing of intron containing genes determines most metazoan mRNA for nuclear export by recruiting certain nuclear export factors exclusively to the spliced transcript (reviewed in Ref. [28]). However, some RNAs in contrast access a different nuclear export pathway by recruiting factors that contain a motif necessary for nuclear export termed nuclear export signal (NES). NES is recognized by the nuclear export receptor CRM-1, a member of the importin/karyopherin superfamily. In the case of retroviral nuclear export, the Rev protein of HIV and Rex protein of HTLV binds to the Rev-responsive elements (RRE) or Rex-responsive elements, respectively. These sequences reside in the introns of unspliced viral RNA, and complex via a NES motif with the CRM-1 protein, thus mediating nuclear export [29,30]. This is the nuclear export pathway used by the RNAs employed in this study. Understanding the nuclear pathway and mechanism of simpler RNA export will aid in the further understanding of how more complex RNAs are produced, processed, and exported in and from nuclei. The presence of Rev and Rex inhibits extensive splicing of the viral RNAs [31,32] and so these RNAs do not require endogenous splicing machinery but are exported rapidly to the cytoplasm. The journey of the RNA from its point of origin to the cytoplasm, through the nucleoplasm, is relatively unchartered. Studies on endogenous gene transcripts do not reveal enough RNA to track their progress and real-time studies involved injected material that may alter nuclear morphology and architecture. Studying excessive amounts of simple viral RNA in the nucleus will reveal aspects of its pathway, behavior and influence of the hosts architecture and organization.

In interphase nuclei, chromosomes are organized as discrete entities termed chromosome territories ([33] and references therein). There appears to be minimal intermingling of chromatin from separate territories [34,35] and a space between chromosomes can be visualized by taking advantage of the assembly properties of the intermediate filament protein vimentin [36–38]. This compartment defined by chromosome surfaces in mammalian cells has been termed the interchromosomal domain compartment (ICD) [9,39]. Previous studies demonstrated that Cajal bodies, PML bodies and specific RNA species lay on the surface of chromosome territories [9,36,40] and that specific coding sequences are distributed at the periphery of chromosome territories and invaginating channels [41–43]. These data have contributed to a model suggesting that transcription occurs at the periphery of a chromosome territory and the subsequent RNA is released into the ICD compartment [9,39]. This model would allow for the nuclear transport of RNA–protein complexes both by diffusion or along pre-existing structures such as filamentous molecules. In the study presented here, we analyzed the spatial nuclear arrangement of individual species of exported RNA to test the concept of a confined RNA distribution in an interchromosomal domain compartment using transiently transfected cells. Furthermore, to elucidate the compartmentalization, synthesis, processing, and transport of transcripts from neighboring genes, RNA derived from co-integrated genes in stably transfected cells was analyzed at high resolution.

Materials and methods

Cells and transfection

Cells were cultured in DMEM containing antibiotics and 10% FCS (v/v). 33-4 is a stably transfected COS-7 cell line.