INTRODUCTION

Transport of RNAs from their transcription sites in the nucleus to their translation sites in the cytoplasm is essential for eukaryotic gene expression. It is generally held that primary gene transcripts associate co- and post-transcriptionally with hnRNPs, snRNPs and probably other factors, for their processing to mRNAs (see for example, Matunis et al., 1993). Also, some of the associated complexes and proteins are believed to assist in the transport of the RNAs through the dense and complex nuclear interior (Izaurralde et al., 1992; Piñol-Roma and Dreyfuss, 1992).

Many of the nuclear constituents are spatially organized within the cell nucleus. Evidence has been provided that chromosomes and components involved in RNA splicing occupy distinct domains in the nucleus (Manuelidis, 1985; Scharin et al., 1985; Lichter et al., 1991; Carmo-Fonseca et al., 1992; Huang and Spector, 1992; Matera and Ward, 1993; Spector, 1993). As a consequence of this spatial organization of nuclear components, nuclear functions such as DNA replication, transcription, RNA processing and possibly nuclear RNA transport are spatially organized (Hozák et al., 1993; Hassan and Cook, 1993; Jackson et al., 1993; Wansink et al., 1993). After or during transcription, RNA splicing is hypothesised as occurring at a latticework composed of interchromatin granule clusters and perichromatin fibrils (Spector, 1993). This high level of organization of the nuclear constituents and processes is thought to be the result of the presence of a nuclear matrix (reviewed by Berezney, 1991; van Driel et al., 1991).

Although RNA molecules are found associated with components of the nuclear matrix (Zeitlin et al., 1987; Xing and Lawrence, 1991), it is still being debated how and along which pathways RNA gene products are transported to the cytoplasm. According to Blobel’s hypothesis (Blobel, 1985), active genes are specifically attached to nuclear pore complexes, and transcripts of such ‘gated genes’ leave the nucleus through the nearby pore complex. This hypothesis has been superseded now, but it did stimulate the search for possible ways of transport of mRNAs in the nucleus.

Visualization of RNA and DNA molecules in intact nuclei is accomplished mainly by fluorescence in situ hybridization (FISH). This technique has in principle a high detection sensitivity, as evidenced by the visualization of unique DNA sequences of 0.5-1 kb, and provides a high spatial resolution compared with chromogenic and radioactive procedures. Thus far, FISH studies with the aim of unravelling the pathway along which gene transcripts are transported to the cytoplasm have not been conclusive. Lawrence et al. (1989) showed that...
transcripts of integrated Epstein-Barr virus (EBV) are localized within a track extending towards the nuclear periphery. Few other studies done since have shown nuclear track-like signals of specific transcripts (Huang and Spector, 1991; Raap et al., 1991; Xing et al., 1993). This led to the prevailing view that RNA transcripts are transported along tracks, representing defined routes from the site of transcription to the cytoplasm. This view has been strengthened further by the immunoelectron microscopic observation of nuclear tracks along which proteins are transported within the nucleus (Meier and Blobel, 1992; Murti et al., 1993). Furthermore, the observation that intron-containing and spliced transcripts of the fibronectin gene are spatially separated in the track suggests that the splicing process is spatially ordered (Xing et al., 1993).

Observations that RNA tracks are not always in contact with the nuclear envelope raised doubt about their function in RNA transport to nuclear pores. It has been speculated that they are accumulation sites for RNA from which processed transcripts may diffuse rapidly away towards the cytoplasm (Lawrence et al., 1993; Xing and Lawrence, 1993). Models have been proposed recently which argue that transcripts are transported to the cytoplasm of cells by means of channelled diffusion through an extrachromosomal channel network or interchromosome domain compartment, which is also supposed to be the site of transcription and pre-mRNA processing (Cremer et al., 1993; Zachar et al., 1993; Zirbel et al., 1993).

Finding answers to the question of how mRNA transport and splicing are organized in the cell nucleus is hampered by the still limited number of active genes studied. Furthermore, RNA-FISH methodology has not been fully validated yet, casting doubt on the reliability of FISH results. Therefore, to provide more data that would contribute to a better understanding of nuclear RNA transport, we decided to investigate the nuclear localization patterns of the human cytomegalovirus immediate early (HCMV-IE) and luciferase gene transcripts in rat 9G and X1 cells, respectively. Both HCMV-IE and luciferase transcripts are expressed in the cytoplasm of these cells. In addition, we studied the nuclear localization pattern of EBV Bam W gene transcripts, of which only a very few are being transported to the cytoplasm of Namalwa cells. Finally, we have analysed methodological aspects with respect to nuclear accessibility and loss of RNA sequences.

From the results obtained at the light microscopical level of spatial resolution, we hypothesise that the nuclear RNA tracks do not represent defined RNA transport routes but, more likely, nascent transcription and/or accumulation of precursor RNA. Also, most of the pre-mRNA processing probably takes place in such tracks. Processed RNA transcripts radiate from this site of transcription and processing to the cytoplasm without following a specific route.

**MATERIALS AND METHODS**

**Cell lines**

Rat 9G cells are transfected rat fibroblasts with a tandem repeat of approximately 10 copies of plasmid pES, which contains the 7.0 kb EcoRI-SalI fragment of HCMV strain AD169 cloned in pBR328 (Boom et al., 1986). This fragment contains a single HCMV-IE transcription unit from which two different HCMV-IE mRNAs, respectively 1.9 kb and 2.1 kb in size, can be derived through differential splicing. The 1.9 kb mRNA codes for the major 72K HCMV-IE nuclear antigen. The cells were grown on glass microscope object slides at 37°C in Dulbecco’s minimal essential medium without Phenol Red (Gibco BRL, Gaithersburg, MD), supplemented with 10% fetal calf serum, antibiotics and glutamine (all from Gibco BRL). X1 cells are HeLa cells with an integrated luciferase gene construct under the control of a tetracycline-controlled, transactivator-dependent promoter (Gossen and Bujard, 1992). The construct consists of the HCMV-IE promoter combined with tetracycline operator sequences, followed by the intron-less luciferase gene fused to the SV40 small-t intron and poly(A) signal. Southern blot analysis revealed that only one to maximally two copies of the luciferase gene are integrated in X1 cells and can be identified in one fragment (Bujard et al., personal communication). The X1 cells were grown on glass slides under the same conditions as the rat 9G cells. Namalwa cells, containing two copies of the EBV genome, were grown in suspension in RPMI with 10% fetal calf serum (Gibco BRL).

**Induction of gene expression**

Expression of the HCMV-IE genes in rat 9G cells was induced by culturing in the presence of 50 μg/ml cycloheximide (Sigma, St Louis, MO) for 4 to 6 hours (Boom et al., 1986). Alternatively, HCMV-IE gene expression was induced by heat-shock, in which case the cells were incubated at 42°C for 15 minutes and allowed to grow for another 4 hours at 37°C. Both treatments resulted in 30% cells showing HCMV-IE mRNA expression.

Luciferase gene expression in X1 cells is sensitive to tetracycline in a concentration-dependent way (Gossen and Bujard, 1992). Transcription of the luciferase gene could be completely blocked by incubating the cells with 1 μg/ml tetracycline in the culture medium. The presence of luciferase activity was checked regularly during maintenance of the cells by a standard firefly luciferase assay (Current Protocols in Molecular Biology, unit 9).

**Cell processing before FISH**

Our in situ hybridization procedure for cultured cells (Raap et al., 1991; Dirks et al., 1993) was slightly modified with respect to cell pretreatment and immunocytochemical detection in order to increase the signal-to-noise ratio of the nuclear hybridization signals. Rat 9G and X1 cells grown on glass microscopic slides were rinsed in PBS and fixed with 3.7% formaldehyde containing 5% acetic acid in PBS for 15 minutes at room temperature.Cells were then rinsed in PBS and stored in 70% ethanol at 4°C. Namalwa cells, or rat 9G cells used in suspension, were rinsed and diluted in PBS, spun onto glass slides using a bucket device (van Driel-Kulker et al., 1980) for 10 minutes at 1,500 rpm and fixed as described above. Cells were then rehydrated in deionized water, incubated with 0.05% or 0.1% (w/v) pepsin (Sigma) in 0.01 M HCl for 1 to 3 minutes, rinsed in deionized water for 30 seconds, washed with 70% ethanol for 1 minute to inactivate peptin, dehydrated in a graded ethanol series and air-dried. The concentration and time of the peptin incubations proved important for maintaining cellular morphology and obtaining signal intensity, and were optimized for each series of experiments.

For some experiments, rat 9G, X1 and Namalwa cells were extracted with Triton X-100 according to the method of Fey et al. (1986). Briefly, rat 9G and X1 cells grown on glass slides were rinsed with PBS for 30 seconds at room temperature. Subsequently, the cells were incubated at 4°C with PBS, cytoskeleton (CSK) buffer (300 mM sucrose, 100 mM NaCl, 5 mM MgCl2, 10 mM PIPES, pH 6.8, 2 mM EGTA, 1 mM phenylmethylsulphonyl fluoride, 2 mM VRC), CSK buffer containing 0.5% Triton X-100, CSK buffer and finally with PBS at room temperature. All incubations were for 30 seconds except for that with Triton X-100, which was varied from 1 to 30 minutes. The most reproducible results leaving cell nuclei intact as judged by DAPI staining were obtained when the cells were incubated for 10 minutes in Triton X-100. Cells were then fixed in 3.7% formaldehyde,
5% acetic acid in PBS for 10 minutes at room temperature, and stored at 4°C in 70% ethanol until use.

Namalwa cells growing in suspension were rinsed in PBS, diluted in CSK containing 0.5% Triton X-100 at 4°C and spun onto glass slides for 10 minutes at 1,500 rpm. Cells were rinsed in CSK buffer and PBS for 30 seconds each and then fixed as described above.

Probes and labelling
Plasmid pSS contains the 5.0 kb SplI-Sall genomic fragment of the transcribed HCMV-IE region (Boom et al., 1986). For the detection of intron- and exon-specific probes, PCR products were made from the intron sequence between exons 1 and 2 and exons 4, respectively, using the primer combinations 123-142 and 903-922 for the intron probe and 1661-1680 and 2581-2600 for the exon 4 probe (numbers indicate positions in the published sequence; Ackrigg et al., 1985). The pGem luciferase probe was purchased from Promega, and the EBV BamHI W fragment-specific probe was made by subcloning it in a pGem vector (Promega). The probe for rRNA detection is a 2.1 kb fragment complementary to the 3′ site of human 28 S rRNA subcloned in a pGem vector. For control experiments plasmids without insert sequences or with an insert encoding the human elongation factor (HEF) housekeeping gene mRNA were used.

Probes were labelled with digoxigenin-12-dUTP (Boehringer Mannheim, FRG) or tetramethyl rhodamine-4-dUTP (Amersham, U.K) by nick translation according to standard procedures.

In situ hybridization
Probes were denatured in a boiling waterbath or denatured simultaneously with target sequences under a coverslip for 3 minutes at 80°C. All probes were used at a concentration of 5 ng/μl hybridization solution (50% formamide, 2× SSC (0.3 M NaCl, 0.03 M sodium citrate), 10 mM EDTA, 25 mM NaH₂PO₄, pH 7.4, 10% dextran sulphate, 250 ng/μl sheared salmon sperm DNA). For double hybridizations, the two differently labelled probes were mixed in the same hybridization solution. Hybridizations were performed at 37°C for 16 hours. After hybridization, cells were rinsed 3 times in 50% formamide, 2× SSC for 10 minutes each at 37°C, and 5 minutes in Tris-buffered saline (TBS: 150 mM NaCl, 100 mM Tris-HCl, pH 7.4) at room temperature. Cells were then incubated with the primary antibody mouse anti-digoxin (Sigma), diluted 1:1,000 in TBS containing 0.5% (w/v) blocking reagent (Boehringer Mannheim) for 45 minutes at 37°C. After several washes in TBS, cells were incubated with rabbit anti-mouse FITC (Sigma) diluted 1:100 for 30 minutes at 37°C, rinsed three times in TBS, and mounted in Vectashield (Vector Labs, Inc) containing 4′,6′-diamidino-2-phenyl indole (DAPI).

Photographs were taken with a Leica DM epifluorescence microscope equipped with single- and dual-band pass filters on 3M 640 ASA colour slide film with exposure times ranging from 20 seconds to 1 minute for fluorescein.

RESULTS

Visualization of IE mRNA transcripts in rat 9G cells
FISH studies of the genomic pSS and exon-specific HCMV-IE probes to formaldehyde/acetic acid-fixed rat 9G cells revealed different stages of expression of the HCMV-IE gene cluster: negative cells, cells with only nuclear signals as well as cells with nuclear and cytoplasmic signals (see also Raap et al., 1991, and Dirks et al., 1993). This variability among cells is in accord with the cell-cycle phase dependency of cycloheximide-induced HCMV-IE expression (Boom et al., 1986). For analysis of nuclear RNA distribution patterns, we selected cells with weak or no cytoplasmic RNA signals because the interpretation of nuclear RNA distribution patterns proved difficult in cells with strong cytoplasmic RNA signals. A main track- or elongated dot-like signal and many small spot-like signals radiating from the main nuclear RNA signal were observed in such nuclei (Fig. 1a). Approximately 10% of nuclei showed track-like signals only. The number of small spots was smaller with the exon probe than with the genomic probe (Fig. 1b). With only a few exceptions, the localization patterns of the small fluorescent spots did not indicate the existence of defined routes or channels by which transcripts are being transported to the cytoplasm. Similar observations were made with the intron-specific HCMV-IE probe (Fig. 1c) and a pBR322 probe (result not shown), but the number of small spots was very much higher. At higher magnification it becomes clear that intron sequences radiate from the transcription site (Fig. 1d). Expression of pBR322 was expected as it has been previously established in nuclear run-on assays that integrated pBR322 sequences are transcribed together with the major HCMV-IE transcription unit in rat 9G cells (Geelen et al., 1987).

Analysis of many tracks after double hybridization with intron- and exon-specific probes revealed complete co-localization (Fig. 1e,f), indicating that primary transcripts are present all along the nuclear track. Further evidence that tracks and elongated dots represent, at least partly, active transcription sites was provided by the observation that transcription sites and RNA tracks were always contiguous, precluding the possibility that tracks represent accumulation sites of transcripts originating from elsewhere in the nucleus.

The tracks or elongated dots were found in 80% of the cells near the nuclear border, directed towards the cytoplasm. Only in about 5% of the track-positive cells were they in direct contact with the nuclear envelope.

The specificity of the hybridizations described above was demonstrated by a number of control experiments (data not shown). First, hybridization signals were absent when HCMV-IE gene expression was not induced by cycloheximide. Integrated HCMV sequences were only visible as a small fluorescent dot in nuclei denatured prior to hybridization, and no signals were observed when the denaturation step was omitted. Second, except for the integrated viral DNA, nuclear hybridization signals were sensitive to RNase treatment. Third, probes specific for sequences that are not being expressed in rat 9G cells gave no hybridization signals.

Though it has been reported that cycloheximide has no effect on transcription by RNA Pol II or Pol III and does not prevent RNA transport, it might have an influence on the localization of transcripts (Kleiman et al., 1993; O’Keefe et al., 1994). To exclude this possible effect on the localization of HCMV-IE mRNA, HCMV-IE gene expression was also induced by a mild heat-shock and the nuclear localization patterns compared. The temperature of the heat-shock treatment was strictly controlled as it has been reported previously that a severe heat-shock inhibits RNA processing (Bond, 1988). Fig. 1g shows that after heat-shock the nuclear localization patterns do not differ significantly from those in cycloheximide-treated cells.

Localization of luciferase transcripts in X1 cells
X1 cells have an integrated luciferase gene construct, the expression of which can be controlled by tetracycline (Gossen and Bujard, 1992). Expression of the luciferase gene was assayed functionally by a firefly luciferase assay and immuno-
cytochemically by the presence of luciferase protein in the cytoplasm of X1 cells (results not shown). RNA in situ hybridization experiments with a plasmid probe containing the luciferase-coding region revealed a heterogeneous pattern of nuclear and cytoplasmic signals when the cells were grown in the absence of tetracycline. Compared with HCMV-IE expression in rat 9G cells, the cytoplasmic signals were generally low in number. The nuclear signal consisted of a bright main fluorescent dot and many small fluorescent spots (Fig. 2a). The small spots clearly emanated from the main dot as their density decreased towards the nuclear periphery, especially in those cells with a regularly shaped nucleus. Some of the nuclei had a lobed appearance. The majority of small nuclear hybridization signals in such nuclei were limited to the lobe harboring the bright main dot (Fig. 2b,c). Elongated dots or tracks were never observed.

Double hybridization experiments with digoxigenin-labelled luciferase probe and a rhodamine-labelled probe specific for 28

Fig. 1. Nuclear distribution patterns of HCMV-IE transcripts in rat 9G cells. FISH with the genomic pSS probe (a) shows many small fluorescent spots radiating from the transcription site (arrow). HCMV-IE exon sequence-specific hybridization (b) reveals fewer fluorescent spots, while similar nuclear distribution patterns are observed with the HCMV-IE intron-specific probe (c, green/yellow fluorescence). The red fluorescence in c shows the simultaneous hybridization to 28 S rRNA via a rhodamine-labelled probe after visualization with a double band-pass filter. At higher magnification (×100), intron sequences are clearly shown to radiate from the nuclear track (d). Bi-colour FISH shows that exon sequences (e, green fluorescence) and intron sequences (f, red fluorescence) co-localize over the full length of the track (arrow). Heat-shock induction of HCMV-IE expression results in a similar distribution of HCMV-IE transcripts to that induced by cyclohexamide (g, FISH with genomic pSS probe; compare with a). Bar, 5 µm.
Transport of nuclear mRNA

S rRNA revealed that luciferase gene transcription was not preferentially localized near nucleoli. Occasionally, active transcription was observed at the border of a nucleolus, and these transcripts, visualized as small fluorescent spots, appear to diffuse around the nucleoli toward the nuclear periphery (Fig. 2d).

The specificity of luciferase mRNA detection was indicated by the absence of hybridization signals in X1 cells cultured in the presence of tetracycline or in non-transfected HeLa cells.

Nuclear transcripts of the EBV Bam W fragment are restricted to a track

In addition to transcripts which are known to be transported to the cytoplasm, where they are present in relatively large amounts, we investigated the nuclear distribution of EBV transcripts in Namalwa cells, very few of which are transported to the cytoplasm (Dambaugh et al., 1986). Hybridization experiments with fluorochromized and haptenized probes specific for the Bam W region revealed strong fluorescent track-like signals in 85% of nuclei, while other nuclei revealed a dot-like signal (see also Lawrence et al., 1989). Most of the tracks extended to the cytoplasm, as could be clearly visualized in cells which were simultaneously hybridized for 28 S rRNA, resulting in a fluorescent signal in the cytoplasm and nucleoli (Fig. 2e).

Besides the track, no, or only a very few, additional fluorescent spots were observed in the nucleoplasm, indicating that EBV Bam W transcripts are ‘trapped’ in the track within the nucleus. Cytoplasmic hybridization signals were not observed, suggesting that the few EBV transcripts present there were not detectable.

Nuclear RNA transcripts are highly accessible for probe hybridization after Triton extraction

The visualization of nuclear transcripts is largely determined by their accessibility to probe sequences and antibodies. To exclude the possibility that nuclear transcripts are not detected because of their poor accessibility, cells were extracted with Triton X-100 before being fixed. A dramatic increase in nuclear hybridization signals was observed when the extracted cells were hybridized with the genomic pSS probe, revealing HCMV-IE transcripts. As well as at the site of transcription, again visualized as a bright, fluorescent, elongated dot or track, fluorescent signals were observed throughout the entire nucleus. Often a gradient was observed from the transcription site to the periphery, with the most spots found near the transcription site (Fig. 3a). In about 20% of the nuclei, the signal intensity was too high to discern a gradient. Also with the HCMV-IE exon-specific probe, a significant increase in the

Fig. 2. Nuclear distribution patterns of gene transcripts in X1 and Namalwa cells. Luciferase mRNAs in X1 cells, visualized as small, green, fluorescent spots, radiate from their site of transcription (a, arrow). In lobed cell nuclei (b, DAPI image), the distribution of luciferase transcripts is restricted to the lobe that harbours the gene (c, arrow). The other nuclear compartments (arrowheads) do not contain luciferase transcripts. Bi-colour FISH (d) shows that transcription sites (green fluorescence) are occasionally located near nucleoli and that transcripts diffuse around them without entering them (double exposure of FISH with digoxigenin-labelled luciferase probe and a rhodamine-labelled 28 S rRNA probe). Bi-colour FISH on Namalwa cells (e) shows that EBV Bam W transcripts (red fluorescence) are confined in a nuclear track which extends towards the cytoplasm (green fluorescence of 28 S RNA hybridization). Nuclei show the blue DAPI counterstain. Bar, 5 μm.
number of hybridization signals was obtained (Fig. 3b). With the intron-specific probe, however, many fewer hybridization spots around the transcription site were observed compared with cells which were not extracted (Fig. 3c, compare with Fig. 1b). The fluorescence intensity of the intron-specific signal at the site of transcription was not influenced by the extraction procedure.

Triton X-100-extracted Namalwa cells did not reveal any, or only a very few, additional fluorescent spots other than the track-like signal of EBV mRNA (Fig. 3d). This shows that mRNA molecules allocated in a track do not diffuse away as a result of the extraction procedure. Furthermore, in agreement with previous observations (Xing and Lawrence, 1991), the fluorescent intensity and shape of the EBV mRNA track were also not influenced.

DISCUSSION

We have analyzed three different genes for their nuclear RNA distribution patterns. Their common characteristic is a very high level of transcription due to the presence of strong (inducible) viral promoter/enhancer elements. In their splicing characteristics and cytoplasmic RNA expression, there are remarkable differences. The EBV transcripts in Namalwa cells and the HCMV-IE transcripts in rat 9G cells are extensively spliced, while in the luciferase transcript in X1 cells only one small intron (66 bp) has to be removed. The EBV transcripts are hardly localized in the cytoplasm (Dambaugh et al., 1986), while the luciferase and HCMV-IE transcripts are abundantly expressed and translated into proteins (Raap et al., 1991; Gossen and Bujard, 1992). These properties may provide us with a clue for a functional interpretation of the nuclear RNA distribution patterns observed.

Table 1 summarizes the FISH results obtained in relation to the characteristics described above. Two correlations are apparent: (1) presence of tracks and extent of splicing; and (2) presence of small nuclear spots and cytoplasmic RNA expression. These correlations might be interpreted as follows. The main nuclear signal represents a domain where transcription and splicing occur. When the extent of splicing is high, as for HCMV-IE and EBV transcripts, there is not sufficient co-transcriptional splicing available to process all the RNA transcripts. Unspliced or partially spliced RNAs begin to occupy track-like domains in the vicinity of the gene or gene cluster, where they are processed post-transcriptionally. This leads to the elongated dot- and track-like signals. As tracks can extend beyond the length of a gene, partially spliced transcripts must have the ability to move a short distance away from the gene (see also Xing et al., 1993). Further transport of the partially

Fig. 3. Nuclear distribution patterns of RNA transcripts in rat 9G (a,b,c) and Namalwa (d) cells extracted with Triton X-100 before fixation. After hybridization with the genomic pSS probe (a) and HCMV-IE exon-specific probe (b), many fluorescent spots are visible in the nucleoplasm in addition to the bright fluorescent transcription site (compare the number of spots in b with those in Fig. 1b). FISH with the HCMV-IE intron probe (c) reveals a strong fluorescent signal (green/yellow) at the transcription site and only a few additional spots in the nucleoplasm, indicating that ‘free’ intron sequences are removed from the nucleoplasm (compare with Fig. 1c). In Namalwa cells (d), no EBV Bam W transcripts are observed other than the main nuclear signal. Nuclei show the blue DAPI counterstain. Bar, 5 μm.
spliced transcripts might be prevented then by associated splicing factors. When the extent of splicing is low, as for luciferase transcripts, splicing is completed co-transcriptionally, leading to a bright dot-like signal. Only spliced products have, in principle, the potential for leaving the main nuclear transcription/pre-mRNA accumulation domain to be transported to the cytoplasm. For the HCMV-IE and luciferase transcripts, the additional small nuclear RNA signals that radiate from the RNA transcription/accumulation domain represent mRNAs in transport to the cytoplasm and spliced-out introns. In this concept the track-like nuclear RNA signals have no function in the transport of RNAs to the cytoplasm.

Although the different distribution patterns are observed in different cell lines, we consider it rather unlikely that these patterns reflect solely differences in cellular physiology. It is plausible to assume that mammalian cells possess a common mechanism by which gene transcripts are being spliced and transported. We can, however, not exclude the possibility that transcription rates of the different genes, and local accumulation of transcripts, are influenced by the integration sites of the gene constructs.

Support for our suggestion that RNA tracks are accumulation sites of abundantly transcribed and partially spliced pre-mRNAs is provided by studies showing a close association of splicing factors with active transcription sites (Huang and Spector, 1991; O’Keefe et al., 1994). Obviously, pre-mRNA molecules do not need to be transported by means of a track to foci containing splicing factors or along a reticular network rich in splicing components where splicing can occur (Spector, 1990).

We can only speculate about the mechanism by which pre-mRNAs are retained in a track. It is most likely a function of the intron sequences, as it has previously been shown that the latter have the ability to maintain and localize pre-mRNAs within the nucleus through their association with spliceosomes (Legrain and Rosbash, 1987; Wang et al., 1991). This suggests the existence of nuclear structures capable of retaining intron-containing transcripts. The physical nature of these structures has not been resolved yet, but they might involve perichromatin fibrils or interchromatin granule clusters, both of which have been known to contain splicing factors (for reviews see Spector, 1993; Fakan, 1994).

Our view of RNA transport, i.e. radial transport of spliced RNAs from the site of transcription to the nuclear periphery, also implies that splicing occurs mainly co-transcriptionally. Despite a number of studies advocating co-transcriptional splicing (Beyer and Osheim, 1988; LeMaire and Thummel, 1990), there is still some controversy over whether or not RNA splicing is indeed completed before RNAs are released from their DNA template (see also Wuarin and Schibler, 1994). Our observation that the number of intron-specific hybridization signals decreases after Triton X-100 extraction while that of exon-specific signals increases as a result of improved accessibility indicates, however, that most of the intron-specific hybridization signals represent spliced-out intron lariats which are less tightly associated with nuclear structures than the exon sequences. It has previously been reported that such a treatment does not influence the localization of nuclear mRNA (Xing and Lawrence, 1991). We as well as others (Xing et al., 1993) did, however, observe a decrease in intron-specific hybridization signals as a result of the extraction procedure.

In conclusion, the mechanism and routes along which transcripts are being transported to the cytoplasm remain puzzling. This study shows that IE and luciferase mRNAs appear to diffuse radially from their site of transcription and processing to the nuclear periphery as indicated by concentration gradients of the two different transcripts extending away from the site at which they are transcribed and processed. This implies that nuclear tracks, as observed in rat 9G cells for HCMV-IE mRNA and Namalwa cells for EBV RNA, do not reflect a transport route but, more likely, are the result of an accumulation of precursor transcripts along chromatin at the site of the transcribed genes.

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REFERENCES


Table 1. Summary of the FISH results obtained in relation to the splicing and cytoplasmic RNA expression characteristics of the transcripts under study

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<th>Extensive splicing</th>
<th>Nuclear track present</th>
<th>Small nuclear dots present</th>
<th>Cytoplasmic expression</th>
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<tbody>
<tr>
<td>HCMV-IE</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Luciferase</td>
<td>–</td>
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<td>+</td>
<td>+</td>
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<td>EBV Bam W</td>
<td>+</td>
<td>–</td>
<td>+</td>
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Probing functional...

Lawrence, J. B., Singer, R. H. and Marselle, L. M. (1989). Some cis- and trans-acting mutants for...


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