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Subcellular Distribution of Human Immunodeficiency Virus Type 1 Rev and Colocalization of Rev with RNA Splicing Factors in a Speckled Pattern in the Nucleoplasm

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The human immunodeficiency virus type 1 (HIV-1) Rev (regulator of virion protein expression) protein exemplifies a new type of posttranscriptional regulation. One main function of Rev is to increase the cytoplasmic expression of unspliced and incompletely spliced retroviral mRNAs from which viral structural proteins are made. In that way, Rev is essential in order to complete the retroviral life cycle. The biology of Rev in the host cell has remained elusive. In this study, a complex distribution of Rev in single cells was found. Rev was found in the cytoplasm, in a perinuclear zone, in the nucleoplasm, and in the nucleoli. In the nucleoplasm, Rev colocalized in a speckled pattern with host cell factors known to assemble on nascent transcripts. Those factors are involved in the processing of heterogeneous RNA to spliced mRNA in the nucleoplasm of all cells. The distribution of Rev was dependent only on Rev and host cell interactions, since neither the Rev target RNA nor other HIV proteins were expressed in the cells. Rev was found in the same subcellular compartments of cells treated for extended periods with cycloheximide, an inhibitor of protein synthesis. This finding implies that Rev shuttles continuously between cytoplasmic and nucleoplasmic compartments. The results suggest a potential role for Rev both in the RNA-splicing process and in the nucleocytoplasmic transport of Rev-dependent HIV mRNA.

The regulators of virion protein expression (abbreviated Rev and Rex in lentiviruses and oncoviruses, respectively) represent a novel class of regulatory proteins first discovered in the retroviruses human immunodeficiency virus (HIV) (Rev) (25, 70) and human T-cell leukemia virus (HTLV) (Rex) (40). The expression of unspliced and incompletely spliced HIV-1 mRNA requires Rev at an unknown step(s) between transcription and translation. The retroviral structural components are made from the Rev-dependent unspliced and incompletely spliced mRNAs. Therefore, mature virions are not formed in the absence of Rev (65, 73). The Rev-dependent mRNAs are characterized by two types of *cis*-acting sequences, a single Rev response element (RRE) (20, 50) and several *cis*-acting repressive sequences (CRSs) (64). These elements are removed in the completely spliced HIV mRNAs which do not require Rev for cytoplasmic appearance and translation. The RRE is present in the HIV *env* region between the two exons of *rev* and folds into a complex stem-loop RNA secondary structure (50) that binds Rev with high affinity (18, 33, 52, 56, 78) in vitro. Mutations that compromise RRE structure abrogate Rev binding and Rev function (21, 36, 41, 52, 56). The CRSs are less well defined and mapped (13, 22, 46, 64, 68) but by definition prevent expression of the HIV-1 mRNAs in which they are present unless counteracted by Rev activity. One hypothesis is that CRSs bind inhibitory host cell structures which are displaced by Rev following its binding to the RRE (15, 32).

Site-directed mutagenesis of the 116-amino-acid (aa) Rev protein has delineated several functional domains (34, 47, 51, 55). Mutations in a basic domain (aa 35 to 50) compromise nuclear and nucleolar accumulation. The basic domain additionally binds specifically to the RRE RNA (14, 47). Other mutations affect Rev multimerization (51, 55). The activation domain (aa 73 to 90), which contains characteristically spaced leucine residues, may generate transdominant negative mutants that repress Rev wild-type function in cotransfection experiments (47, 51, 74). It has been reported that the activation domain ends with critical acidic residues (53).

Different experiments have suggested that the splicing apparatus (10, 45), the RNA transport system (22, 26, 31, 50), and the cytoplasmic polysomes (1, 17) are potential targets for Rev activity. Morphological studies have shown that Rev is imported to the nucleus and accumulates in the nucleolus (14, 16). To study Rev in the context of the single cell, a panel of monoclonal antibodies (MAbs) was raised against recombinant Rev expressed in *Escherichia coli* and selected for reactivity in indirect immunofluorescence in Rev-expressing cells. Rev was distributed to the cytoplasm, to a perinuclear zone, to the nucleoli, and to the nucleoplasmic speckles believed to be sites of processing of RNA polymerase II pre-mRNA. The continuous shuttling of Rev between the different subcellular compartments supports a model in which the role of Rev is both to interfere with splicing of HIV mRNA and to be involved in HIV mRNA transport and in cytoplasmic events of HIV mRNA expression.

MATERIALS AND METHODS

Plasmid constructs. Plasmid pSVCMV *rev* contains the HIV-1 IIIB *rev* cDNA (*Bam*HI-*Eco*RI fragment derived from pH3*art* [64]) downstream of the simian virus 40 origin and the cytomegalovirus promoter and upstream of the simian virus 40 poly(A) signal in a pGEM backbone. In p*rev* 81 (74), the *rev*

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cDNA mRNA transcription is driven by the cytomegalovirus immediate-early promoter. pSVβgal was from Promega Biotec.

Cell lines and transfection. COS-1 cells were transfected by using standard DEAE-dextran and calcium phosphate methods, with the same results. One day following transfection, cells were trypsinized, plated on 12-mm-diameter Assistant coverslips, fixed in methanol between 36 and 72 h posttransfection, and examined. HeLa/*crev*8 cells and HeLa/*cnef* cells (gifts from Bryan Cullen, Howard Hughes Medical Institute, Durham, N.C.) were made essentially as described for HeLa/*ctat* cells (48). In HeLa/*crev*8 cells, *rev* is expressed from the full-length *rev* cDNA present in p*crev* (49). HeLa/*crev*8 cells were grown in Iscove's medium (GIBCO) supplemented with fetal calf serum and 5×10^{-7} M methotrexate.

Autoimmune sera and MAbs. The human autoimmune sera used recognize nucleoplasmic speckles (Sigma and Chemicon), nucleoli (ANA-N; Sigma), the mitotic spindle (Chemicon), and lamin A/C (a gift from Frank McKeon, Harvard Medical School, Boston, Mass.). The sera were characterized by fluorescent double labeling with MAbs of known specificity. The autoimmune antilamin serum colocalized with an anti-lamin A/C MAb (Frank McKeon). MAb anti-SC35 (28) detects an essential non-small nuclear ribonucleoprotein (snRNP) RNA-splicing factor localized in nucleoplasmic speckles. MAb Y12 (44) recognizes the sm antigen common to U1, U2, U4/U6, and U5 snRNPs which all are present in speckles. MAb La1B5 detects the La antigen (RNA polymerase III termination factor and a nucleocytoplasmic shuttle protein [4]). MAb anti-B″ (30) detects the U2 snRNP-specific B″ protein in the speckles. H20 (6, 61) detects the specific modification of snRNAs, 2,2,7-trimethylguanosine (m_3G caps) present in the speckles. Antibodies against heterogeneous nuclear ribonucleoproteins (hnRNP) A1 (4B10) and C (4F4) (12) were used. Other control MAbs were anti-B23 (54) and anti-C23 (27), directed against major nucleolar antigens involved in nucleocytoplasmic shuttling, and MAbs against nuclear pore complexes (RL-1 [69] and MAb350 [19]) and an anti-rRNA MAb (44). Anti-cyclin A and anti-cyclin B1 were from Pharmingen. An anti-C-terminal Rev polyclonal rabbit serum was a gift from M.-L. Hammarskjöld and David Rekosh (State University of New York at Buffalo, Buffalo, N.Y.).

Immunofluorescence analysis. Coverslips with cells were washed twice in phosphate-buffered saline (PBS) and fixed in ice-cold methanol for at least 10 min. Following two washes in PBS, the coverslips were incubated in 0.5% bovine serum albumin (BSA) in PBS for 15 min. The first antibody at appropriate dilution in PBS–and 0.5% BSA was added for 1 h. PBS washes were done before and after incubation with Texas red- or fluorescein isothiocyanate (FITC)-conjugated secondary antibodies for 0.5 h. FITC anti-human (Boehringer), FITC anti-mouse (Boehringer), and Texas red anti-mouse (Amersham) conjugates were diluted 1:100 in 0.5% BSA in PBS.

Generation and characterization of anti-Rev hybridomas. Three BALB/c mice were immunized with 50 μg of purified recombinant HIV-1 Rev expressed in *E. coli* (American Biotechnology, Boston, Mass.) in a volume of 500 μl with 50% Freund's complete adjuvant. Three boosts of 50 μg of Rev in PBS and Freund's incomplete adjuvant and a fourth boost of 50 μg of Rev in PBS were given at monthly intervals. Three days following the last boost, the spleen was removed and the splenocytes were fused with the myeloma cell line NSO by standard methods using polyethylene glycol 4000. The initial screening of antibody producing hybridomas was done by enzyme-linked immunosorbent assay, using 100 ng of Rev per well. Indirect immunofluorescence of Rev-expressing cells was used for the selection of hybridomas that generated strong fluorescent signals. The selected hybridomas were cloned by fixed dilutions until monoclonal cultures were obtained. Four different hybridomas, 9G2, 8E7, IG10, and 1G7, worked well in indirect immunofluorescence and also detected Rev in Western immunoblot assays. The MAbs were mapped by using a panel of 14 overlapping synthetic peptides spanning the whole length of the Rev protein (ABT, USA). IG10 and 1G7 reacted specifically with a synthetic peptide corresponding to Rev aa 96 to 105 (GVGSPQILVE), and 8E7 and 9G2 reacted with a peptide corresponding to Rev aa 70 to 84 (PVPLQLPPLER LTLD).

A kit (Zymed) was used for the isotyping of antibodies. 9G2 and 8E7 both belonged to the immunoglobulin G2a kappa subtype, and IG10 and 1G7 belong to the immunoglobulin G2b kappa subtype.

Synchronization and arrest of the cell cycle of HeLa/*crev* cells. The procedure used was essentially that described previously (11). HeLa/*crev* cells were grown in Iscove's medium supplemented with 1% fetal calf serum for 2 days. G_0-arrested cells were exposed to aphidicolin at 10 μg/ml in complete medium for 12 to 15 h. Cells arrested by aphidicolin in the G_1/S stage of the cell cycle were released by two washes and incubation in complete growth medium. The cells were monitored by the number and appearance of mitotic spindles, using a human anti-mitotic spindle serum (Chemicon).

RESULTS

Selection of hybridomas producing anti-Rev MAbs strongly reactive in indirect immunofluorescence. A panel of hybridomas secreting anti-Rev MAbs was generated. Four of twelve hybridomas that specifically detected Rev in a Western blot analysis reacted strongly in immunofluorescence with HeLa/*crev* cells and showed no background staining of HeLa/*cnef* cells. These antibodies (9G2, 8E7, IG10, and 1G7) generated the same fluorescent patterns, although they mapped to at least two distinct epitopes of Rev. The eight other antibodies either did not generate detectable fluorescence in HeLa/*crev* cells or generated nonspecific labeling by staining of cells that did not express Rev.

Specificity of the anti-Rev MAbs. The different anti-Rev MAbs specifically detected Rev in cell lysates of HeLa/*crev* cells, cells transiently transfected with pSVCMV*rev*, and HIV-1 IIIB-infected C8166 cells, using both a radioimmunoprecipitation assay and Western blotting. The fluorescent patterns of Rev detected with the anti-Rev antibodies 8E7, 9G2, IG10, and 1G7 were present only in Rev-expressing cells like HeLa/*crev* cells (Fig. 1) and COS-1 cells transfected with the *rev* expressor plasmids pSVCMV*rev* (Fig. 2) and p*rev* 81 (74) (not shown). HeLa/*cnef* cells and mock-transfected COS-1 cells did not stain with the anti-Rev antibodies when used in parallel experiments (not shown). Cells transfected with pSVβgal (Promega Biotec) expressed β-galactosidase in 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) color substrate assay (62) but did not stain with the anti-Rev antibodies. The various FITC, rhodamine, and Texas red conjugates used in the absence of the anti-Rev antibodies or other primary antibodies or sera used in this study generated only a faint background fluorescence, in contrast to the parallel experiments including the primary antibodies.

Patterns of Rev accumulation revealed by the anti-Rev MAbs. The anti-Rev MAbs visualized several new subcellular localizations of Rev (Fig. 1 and 2). In addition to the previously recognized nucleolar (Fig. 1C, E, and G and 2A) and diffuse nucleoplasmic (Fig. 1G) accumulation, Rev was detected as

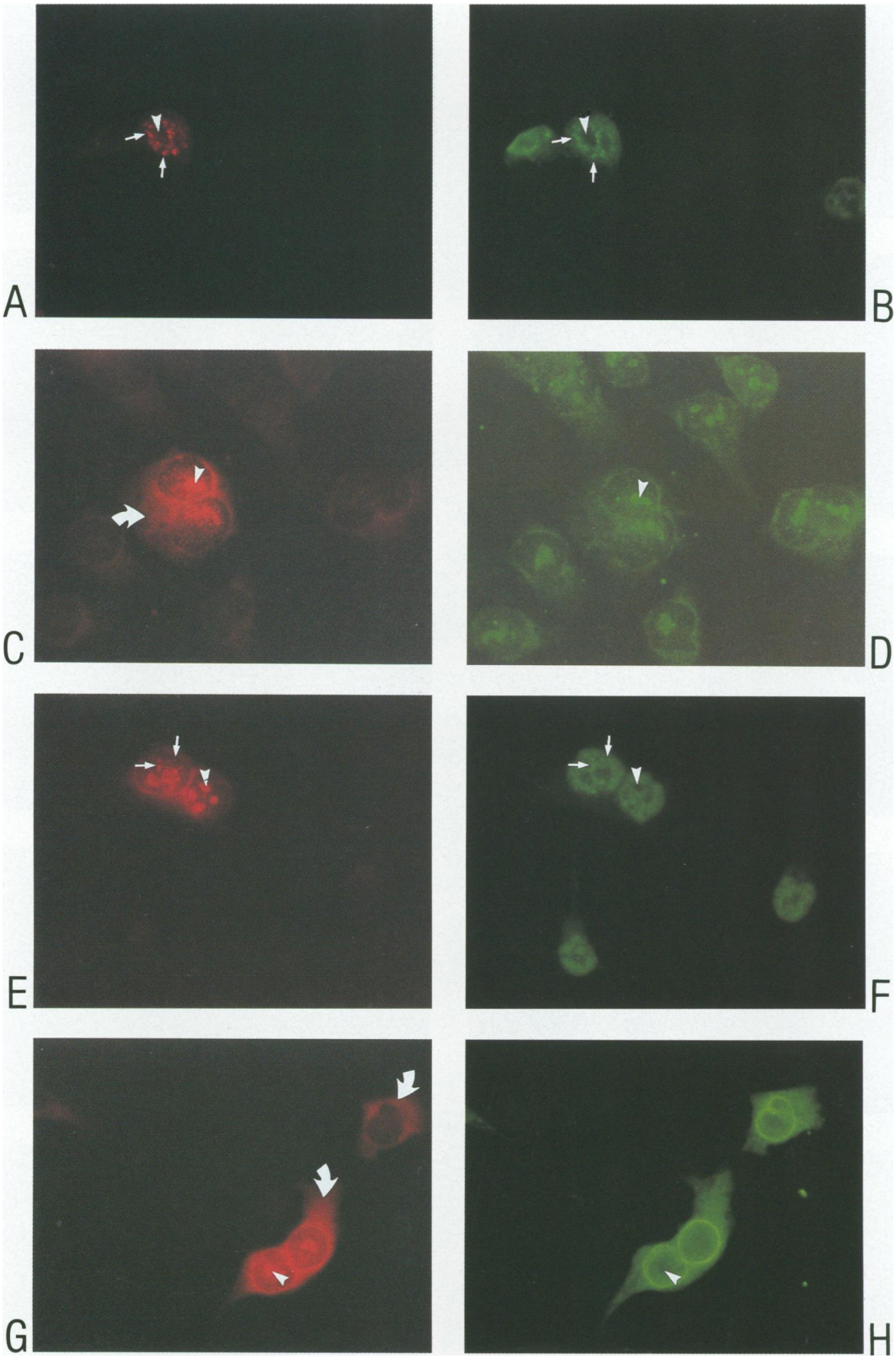

FIG. 1. Indirect immunofluorescent double labeling of HeLa/*crev* cells showing Rev in the snRNP-rich nucleoplasmic speckles (small arrows), in the nucleoli (arrowheads), in a perinuclear zone, and in the cytoplasm (curved arrow). Left-right pairs of panels represent the same microscopic field (magnification, ×400). (A, C, E, and G) Anti-Rev MAb 9G2 and a Texas red anti-mouse conjugate; (B and F) human autoimmune sm-positive serum; (D) human autoimmune serum against nucleoli, ANA-N; (H) human autoimmune serum against lamins. The human antisera in panels B, D, F, and H were visualized by using an FITC anti-human conjugate.

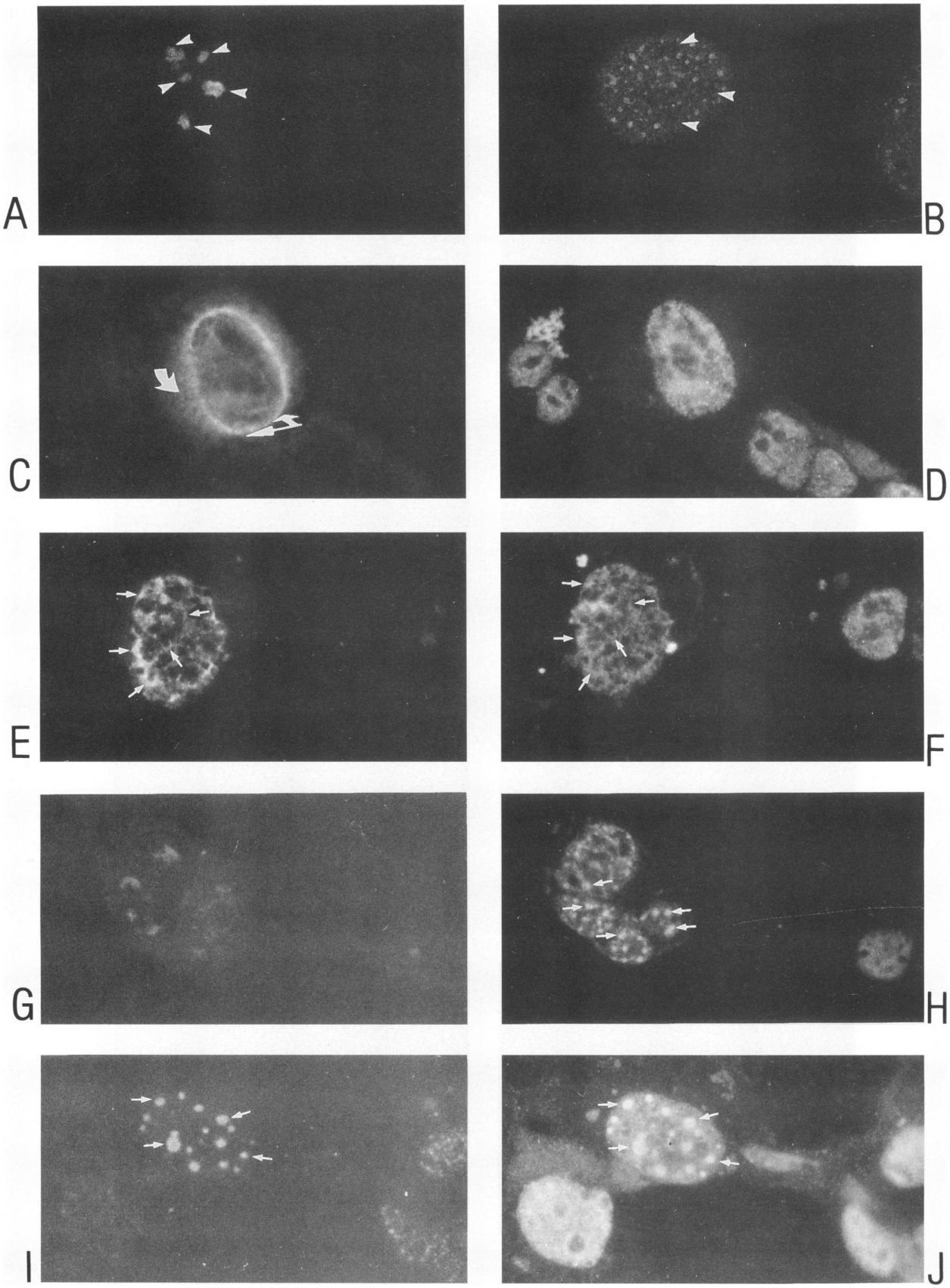

FIG. 2. Indirect immunofluorescent double labeling of COS-1 cells transfected with pSVCMV*rev* and fixed 64 h later. Left-right pairs of panels represent the same microscopic field (magnification, ×600). (A, C, E, and G) Anti-Rev MAb 9G2 and a Texas red anti-mouse conjugate; (I) anti-SC35 MAb and a Texas red anti-mouse conjugate; (B, D, F, H, and J) human sm-positive serum (speckled) and an FITC anti-human conjugate. Shown are the nucleoli (arrowheads), perinuclear zone (split arrow), cytoplasm (curved arrow), and localization of nucleoplasmic snRNPs (small arrows).

TABLE 1. Colocalizations between Rev and host cell factors, determined by indirect immunofluorescent double labeling

MAb or serum (antigen recognized)	Colocalization[a]			
	Nucleoplasmic speckles		Perinuclear zone	
	sm serum	Rabbit anti-Rev	sm serum	Rabbit anti-Rev
MAb				
8E7 (HIV-1 Rev)	+	+		+
9G2 (HIV-1 Rev)	+	+		+
4B10 (hnRNP A1)	+	+		−
4F4 (hnRNP C)	+	+		−
H20 (m_3G caps)	+	+		−
Y12 (sm antigen)	+	+		−
Anti-B″ (U2 snRNP B″)	+	+		−
Anti-SC35 (SC35)	+/−	+/−		−
Antilamin (lamin A/C)	−	−		−
MAb350 (nuclear pore)	−	−		−
RL-1 (nuclear pore)	−	−		−
La1B5 (La antigen)	−/+	−/+	−	−/+
Anti-B23 (B23)	−	−	−	−/+
Anti-C23 (nucleolin)	−	−	−	−/+
Anti-rRNA (rRNA)	−	−	−	−/+
Serum				
Sm positive (sm antigen)	+	+		−
ANA-N (nucleolus)	−	−	−	+
Antilamin (lamin)	−	−		−

[a] +, both antibodies of the corresponding coordinates label the nucleoplasmic speckles or the perinuclear zone; −, only one of the two antibodies labels the speckles or the perinuclear zone; +/−, considerable double labeling; −/+, some double labeling.

distinct speckles within the nucleoplasm (Fig. 1A, C, and E and 2E) and additionally in a perinuclear zone (Fig. 1C, E, and G and 2C) from which Rev-containing rays sometimes extended into the cytoplasm (Fig. 2C). Rev was also diffusely distributed in the cytoplasm of many cells (Fig. 1C and G and 2C). In some cells, mainly the nucleoli stained (Fig. 1E and 2A); in others, mainly the nucleoplasmic speckles (Fig. 1A and 2E), the perinuclear zone (Fig. 2C), or the cytoplasm (Fig. 1G, upper right) stained. Any combination of those basic accumulations was found. In repeated experiments of nonsynchronized cultures, the prevalence of the basic subcellular distributions of Rev was comparable.

Characterization of human autoimmune sera by fluorescent double labeling. Sm-positive sera generate a speckled nucleoplasmic immunofluorescent staining, indicating that mRNA splicing components are nonrandomly distributed in the nucleoplasm (44). MAbs against the sm antigen of snRNPs (44), RNA-splicing factors like U2AF (77) and SC35 (28), and some hnRNPs (12, 23, 59) generate a similar speckled nucleoplasmic immunofluorescent staining. Additionally, poly(A) RNA (9), m_3G cap RNA (6, 61), U1, U2, and U5 snRNAs (8), and specific mRNA transcripts (35, 75) have been localized to similar nucleoplasmic speckles. Whether distinct subsets of speckles exist is still unclear. The functional relationships between speckles and a number of morphological distinct, subnuclear domains including foci or coiled bodies (8, 43, 60, 79), hnRNP L domains (8, 59), nuclear dots (3), and PIKAs (66) await elucidation.

This study will deal with speckles as the fluorescent pattern generated by the sm-positive autoimmune human serum. The speckled immunofluorescent colocalizations between defined subcellular components are shown in Fig. 3 and Table 1. In methanol-fixed cells, antibody Y12, directed against the sm antigen, completely colocalized with the speckles generated by the sm-positive human serum. In addition, both stained the nucleoplasm diffusely (Fig. 3A and B). Extensive colocalization between the speckles of the sm-positive serum, the m_3G caps, the U2 snRNP-specific protein B″, hnRNP A1 (Fig. 3C and D), and hnRNP C (Fig. 3E and F) was found. In some preparations, a partial colocalization between the sm-positive serum and the La1B5 antibody (4), directed against the La antigen, was found (Fig. 3G and H). The SC35 staining was sometimes different from the sm staining, consisting of larger, irregular structures enriched around the nucleolus (Fig. 4H). The extent of colocalization between the SC35 staining and the sm-positive staining surprisingly appeared variable in methanol-fixed cells in repeated experiments.

HIV-1 Rev associated with splicing factors in the nucleoplasm. The autoimmune sera reacting with the same nucleoplasmic speckles as MAbs to spliceosomal components were used in combination with the anti-Rev MAbs. Colocalization between Rev and the nucleoplasmic speckles is evident in Fig. 1A, B, E, and F and Fig. 2E and F. Almost all cells stained positive with autoimmune sera or MAbs against subcellular structures. In comparison, a minority of the same cells stained strongly with the Rev antibodies (Fig. 1, 2, and 4). Only the autoimmune serum with the nucleoplasmic speckled reactivity colocalized with the nucleoplasmic Rev speckles. Neither the human antinucleolar (Fig. 1C and D) nor the human antilamin (Fig. 1G and H) serum double stained the nucleoplasmic Rev spots.

In double-labeling experiments using 9G2 or 8E7 and a polyvalent rabbit antiserum directed against the Rev C-terminal part, the same HeLa/*crev* cells stained with overlapping fluorescent patterns (Fig. 4A and B). The results of the fluorescent double labeling using the polyclonal anti-Rev serum and MAbs against defined subcellular components are displayed in Table 1 and Fig. 4. Extensive colocalization in nucleoplasmic speckles between Rev and B″ (Fig. 4C and D), the sm antigen (Fig. 4E and F), and hnRNP A1, hnRNP C, and m_3G caps (Table 1) was evident. The colocalization between Rev and SC35 appeared sensitive to slight variations of the immunofluorescence procedure and varied (Fig. 4G and H). In less than 10% of Rev-positive cells, significant speckled colocalization between Rev and La1B5 (Fig. 4I and J) existed.

Rev accumulates in a perinuclear zone enriched in other host cell antigens that are transported across the nuclear membrane. A large proportion of Rev-expressing HeLa and COS cells exhibited a pronounced perinuclear accumulation of Rev (Fig. 1G and 2C). In some cases, Rev-containing rays extended beyond the zone and into the cytoplasm (Fig. 2C). The zone was outside the areas that were stained by both the antispeckled serum (Fig. 2C and D) and the antilamin serum (Fig. 1G and H). Double labeling between anti-nuclear pore MAbs (RL-1 and MAb350) and the rabbit anti-Rev (not shown) did not clearly resolve the extent of colocalization between the nuclear membrane and the perinuclear zone. From FITC and Texas red overlays (not shown) and conventional fluorescence microscopy, it seemed that the Rev zone did not overlap with the nuclear part of the nuclear membrane but overlapped or was contiguous to the cytoplasmic side of the nuclear membrane. Several characterized antibodies stained the Rev perinuclear zone, including the La1B5 antibody (Fig. 4I and J) and MAbs against the major nucleolar proteins, B23 and C23 (not shown). Notably, all of these proteins belong to a growing class of proteins known to shuttle between the nucleus and the cytoplasm.

The different Rev staining patterns did not vary in subclones of the HeLa/*crev* cell culture or with the abundance of Rev in

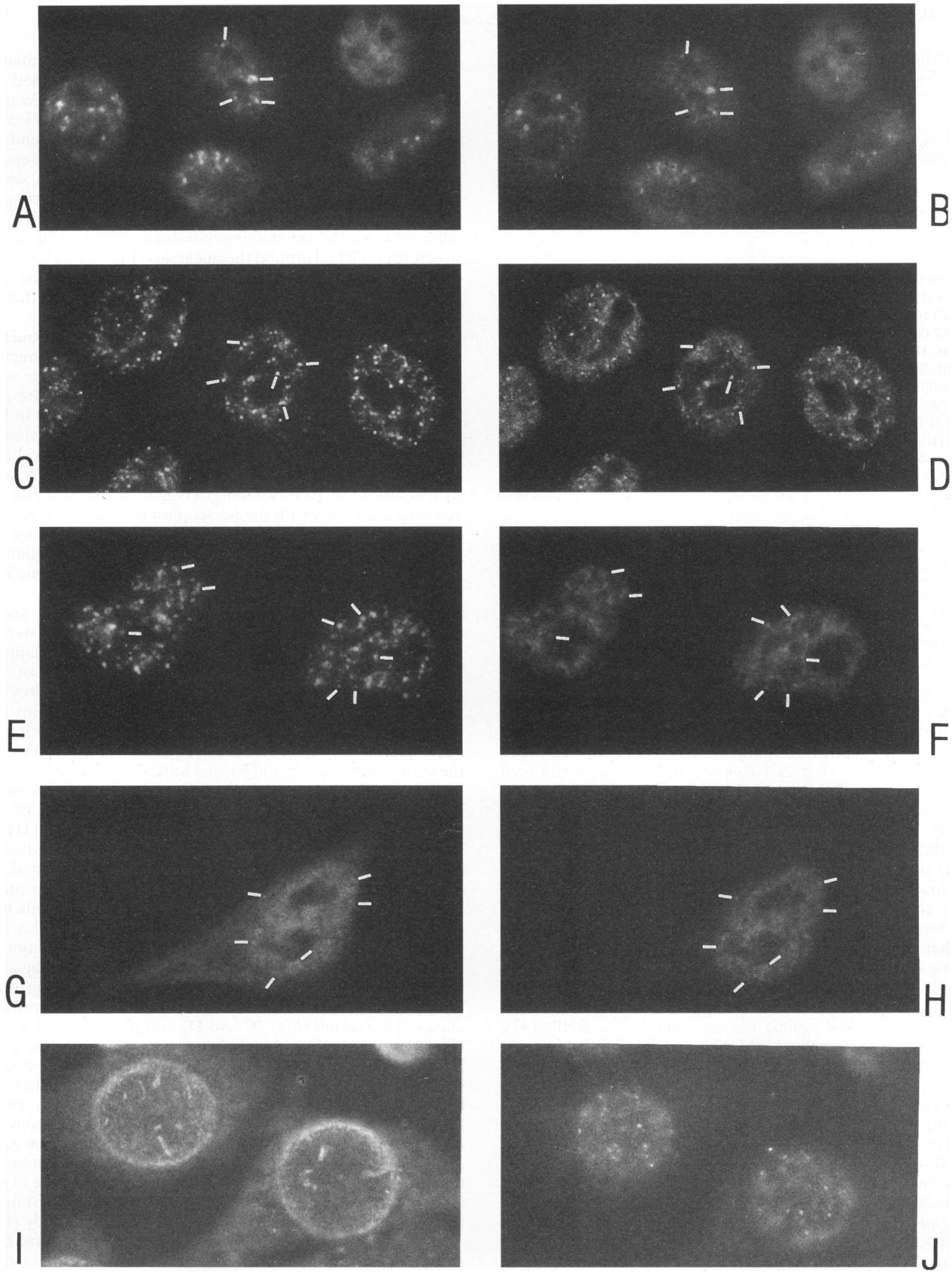

FIG. 3. Characterization of the human sm-positive serum (Chemicon) in panels B, D, F, H, and J, using indirect immunofluorescent double labeling of HeLa/*crev* cells with MAbs Y12 (A), 4B10 (C), 4F4 (E), La1B5 (G), and MAb350 (I). The sm antibodies were visualized with rhodamine anti-human conjugates, and the MAbs were visualized with FITC anti-mouse conjugates. See Table 1 for results and more extensive characterization performed with additional antibodies. Some of the double-labeled speckles are indicated (bars).

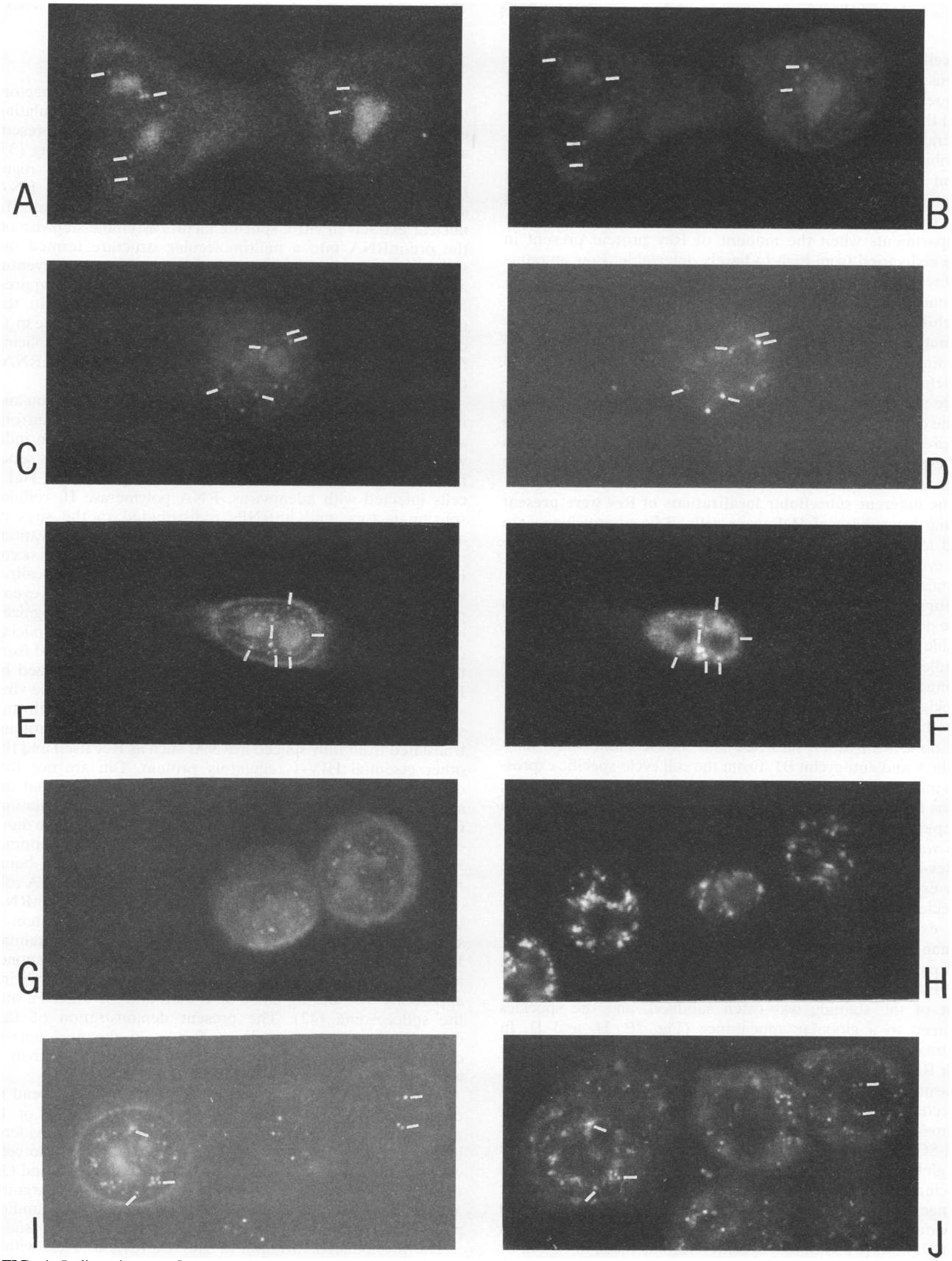

FIG. 4. Indirect immunofluorescent double labeling of HeLa/*crev* cells. All cells in panels A, C, E, G, and I were labeled with a polyclonal rabbit anti-Rev serum and a Texas red anti-rabbit conjugate. In panels on the right, the following MAbs and an FITC anti-mouse conjugate were used: 8E7 anti-Rev (B), anti-B″ (D), Y12 (F), anti-SC35 (H), and La1B5 (J). Some of the double-labeled nucleoplasmic speckles are indicated (bars).

the cells. To examine the possibility that a series of subpopulations of Rev-expressing cells accounted for distinct subsets of unique Rev distributions, many new subclones were grown from the original HeLa/*crev*8 cells. In all of the subclones that generated positive Rev fluorescence, all of the original Rev distribution patterns reappeared (not shown). All of the different HeLa/*crev* clones showed a pronounced cell-to-cell variation in the amount of Rev expression. Rev did not preferentially accumulate in any of the particular subcellular compartments when the amount of Rev protein present in each cell varied from high to barely detectable. Low amounts of Rev were visible in the cytoplasm only (Fig. 1C and G), in the nucleoli (Fig. 1C), and in the speckles only (Fig. 1A).

Inhibition of new protein synthesis by using cycloheximide did not change the subcellular distribution patterns of Rev. To examine whether a final destination of Rev accumulation could be defined, the HeLa/*crev* cells were incubated with cycloheximide at 50 μg/ml to inhibit new synthesis of protein. As long as the cells appeared reasonably healthy, for up to 8 to 10 h in the presence of cycloheximide, the distribution of Rev between the cytoplasm, the perinuclear zone, the nucleoli, and the nucleoplasmic speckles continued.

The different subcellular localizations of Rev were present during interphase of HeLa/*crev* cells. Two approaches were used to determine the relationship between the stage of the cell cycle and the Rev distribution patterns. Cells were synchronized by serum starvation and next treated with aphidicolin for arrest in the G_1/S stage of the cell cycle. Time points were done for cells released from aphidicolin arrest. Cells were double stained by anti-Rev MAbs and a human anti-mitotic spindle serum. Rev was detected in the cytoplasm, in the perinuclear zone, in the nucleoli, and in the nucleoplasmic speckles in G_1, S, and G_2 stages (not shown).

In the second approach, immunofluorescent double labeling was done with rabbit anti-Rev and mouse monoclonal anti-cyclin A and anti-cyclin B1. From the cell cycle-specific expression and subcellular localization of cyclin A and cyclin B1 (57), it was concluded that Rev was expressed and distributed with comparable patterns throughout the interphase (not shown). Rev was also detected in mitotic cells.

Rev-induced change of speckled morphology in vivo. In the Rev-expressing COS-1 cells, two types of changes of the speckled staining pattern were observed. In 60 to 80% of Rev-expressing cells with Rev present in the nucleoplasm, the human antispeckled serum stained the nucleoplasm in a reticular pattern. Rev closely colocalized with the reticles (Fig. 2E and F). In the other Rev-expressing cells, the diffuse component of the staining was often subdued, and the speckles changed to a globular appearance (Fig. 2B, H, and J). In contrast to the reticles, the globules usually did not colocalize with Rev. Instead, Rev was present as crescents in the nucleoplasm between the globules (Fig. 2G and H), or Rev was not detected in the nucleoplasm but was detected in the other compartments (Fig. 2A and B). Double labeling showed that anti-SC35 (Fig. 2I/J) and the Y12 MAb (not shown) stained the globules. In Fig. 2I and J, the extreme size that the changed splicing structures sometimes evidenced is shown. A similar change of the staining of the sm antigen has been reported previously and has been associated with inhibition of mRNA transcription (5). Globular speckles such as those in Fig. 2I or the intense reticular Rev staining such as that in the Rev-expressing COS cells in Fig. 2F were not found in HeLa/*crev* cells and mock-transfected COS cells.

DISCUSSION

We found that Rev colocalizes with mRNA-splicing factors in a speckled pattern in the nucleoplasm of cells. Accumulating evidence shows that the nucleoplasmic speckles represent active sites of both transcription and mRNA processing (37, 38, 76). A long-standing unsolved issue is whether Rev regulation of HIV mRNA expression is at the level of RNA splicing, RNA transport, or other posttranscriptional steps. In nuclear extracts in vitro, splicing factors assemble stepwise on the pre-mRNA into a multimolecular structure termed the spliceosome, from which correctly spliced mRNAs are eventually released (reviewed by Green [29]). The elements required for RNA splicing in vitro are located in speckles in the nucleoplasm that include snRNP proteins (61, 71, 72), the m_3G caps of snRNAs (61), specific snRNAs (8), the RNA-splicing factor SC35 (28), poly(A) RNA (9), and specific pre-mRNAs (35, 75).

Recently, by using a combination of in situ hybridization and immunofluorescence (76), it was demonstrated that introns and exons of specific mRNA transcripts emerged from individual SC35-containing speckles. The introns and exons separated along a track emanating from those speckles. In HeLa cells infected with adenovirus, RNA polymerase II, cellular splicing factors, and hnRNPs redistributed to the sites of adenovirus mRNA transcription (38). A series of observations now suggests that transcription and RNA-splicing events occur in or close to the nucleoplasmic speckles that Rev colocalizes with. Splicing of retroviral mRNAs is a rate-limited event, probably due to splice sites that are suboptimal as recognized by the splicing machinery (10). A complex differential splicing (67) of the approximately 9-kb mRNA that is transcribed from the HIV-1 genome is one of the main strategies utilized by HIV-1 to express its condensed genetic information. The viral structural products that are encoded by the full-length and singly spliced mRNAs are Rev dependent, while proteins translated from fully spliced mRNAs such as Rev itself and the other essential HIV-1 regulatory protein, Tat, are not Rev dependent. Therefore, it should come as no surprise that the splicing process may be a target for retroviral regulation. Chang and Sharp (10) have shown that if splicing is made more efficient by substituting β-globin splice sites for the suboptimal HIV splice sites, Rev cannot rescue the intron from being removed by splicing although the RRE is present in *cis*. A role of U1 snRNA in the formation of Rev-dependent *env* mRNA has been demonstrated (45). In a cell-free splicing system, a small Rev-dependent inhibition of splicing of RRE-containing RNA has been demonstrated. A synthetic peptide corresponding to the basic domain of Rev significantly inhibited splicing apparently by blocking entry of U4/U6 and U5 snRNPs into the spliceosome (42). The present demonstration of Rev colocalization with the splicing factors in the nucleoplasm corroborates the view that one strategic site of Rev activity is within the splicing machinery of the cell.

It might be argued that overexpression of Rev can lead to oversaturation of the host cell transport apparatus or to nonspecific binding of Rev to host cell RNA. It was evident, however, that barely detectable amounts of Rev in the cells were specifically distributed to the cytoplasm (Fig. 1C and G), the nucleoli (Fig. 1C), and the speckles (Fig. 1C), arguing against the possibility that overexpression is a confounding parameter leading to artificial Rev distributions in this study.

The pronounced variation of Rev expression levels within single cells of the same culture (Fig. 1 and 4) could not be related to different stages of the cell cycle and was apparent in newly established subclones of the HeLa/*crev* culture. Most

likely, the varying Rev expression can be attributed to unstable control mechanisms for the artificially introduced *rev* gene. The wide range of abundance levels of Rev in individual single cells show that even when the amount of Rev within the cell is very low, Rev is specifically transported into both the nucleoli and the speckles. Notably, the RRE is made neither in the HeLa/*crev* cells nor in the Rev-expressing COS-1 cells examined, nor is any other HIV-1 protein expressed. It can therefore be concluded that the initial distribution of Rev within the host cell is based entirely upon Rev and host cell interactions. Rev must therefore contain the information not only for nuclear and nucleolar import but also for the RRE-independent localization to nucleoplasmic speckles.

It has previously been shown that Rev promotes the nuclear-to-cytoplasmic distribution of RRE RNA (22, 26, 31, 50). In one study of the analogous HTLV-I Rex protein, it was shown that Rex affects not only the nuclear-to-cytoplasmic distribution of Rex-dependent RNA but also the intranuclear distribution of Rex-dependent RNA (39). The demonstration of Rev-dependent polysomal loading of RRE RNA is consistent with the possibility that Rev has both nuclear and cytoplasmic functions (1, 2, 17). In the present study, it was found that when protein synthesis was inhibited by cycloheximide for up to 10 h, Rev continued to accumulate unabated in the different subcellular compartments. It has previously been shown that nucleocytoplasmic protein transport is not inhibited by cycloheximide under similar conditions (5, 7, 58). The results show that there is no preferred final destination of Rev accumulation. Instead, a continuous redistribution of Rev between the subnuclear and cytoplasmic compartments occurs.

Several host cell proteins that shuttle between the nucleus and the cytoplasm (B23, C23, and La) colocalized with Rev in the perinuclear zone. A similar perinuclear zone has previously been identified as a translationally active ribosome-rich compartment (5); a similar zone has also been associated with the passive attachment stage of the active protein import through the nuclear pore complex (63).

The role of the nucleolus remains enigmatic, since this structure has no known role in processing or transport of RNA polymerase II-transcribed mRNA, the presumed target for Rev when the RRE is present in those mRNAs as well. One possibility is that Rev exploits the high protein import capacity of the nucleolus to penetrate the nucleus. It has been found that Rev binds specifically to a major nucleolar protein, B23 (24), that shuttles between the cytoplasm and the nucleus (7). In this study, colocalization between Rev and B23 was found both in the nucleolus and in the perinuclear zone.

This study has identified new subcellular compartments of Rev distribution. The RRE-independent and continuous distribution of Rev between the cytoplasm, the nucleoli, and the nucleoplasmic speckles supports the following model of the Rev mechanism of function. In the speckles, Rev may dissociate assembly of splicing factors on nascent HIV mRNA transcripts. By dual binding to the RRE of Rev-dependent mRNAs and to host cell factors involved in nucleocytoplasmic transport, Rev may mediate transport of such mRNAs to cytoplasmatic translation.

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REFERENCES

1. **Arrigo, S. J., and I. S. Y. Chen.** 1991. Rev is necessary for translation but not cytoplasmic accumulation of HIV-1 *vif*, *vpr* and *env/vpu* 2 RNAs. Genes Dev. **5**:808–819.
2. **Arrigo, S. J., S. Heaphy, and J. K. Haines.** 1992. In vivo binding of wild-type and mutant human immunodeficiency virus type 1 Rev proteins: implications for function. J. Virol. **66**:5569–5575.
3. **Ascoli, C. A., and G. G. Maul.** 1991. Identification of a novel nuclear domain. J. Cell Biol. **112**:785–795.
4. **Bachmann, M., W. J. Mayet, H. C. Schröder, K. Pfeifer, K.-H. Meyer zum Büschenfelde, and W. E. G. Müller.** 1986. Association of La and Ro antigens with intracellular structures in HEp-2 carcinoma cells. Proc. Natl. Acad. Sci. USA **83**:7770–7774.
5. **Bachmann, M., H. C. Schröder, D. Falke, and W. E. G. Müller.** 1988. Alteration of the intracellular localization of the La protein compared with the localization of U snRNPs, p. 101–125. *In* R. Peters (ed.), Nucleocytoplasmic transport. Academic Press, San Diego, Calif.
6. **Bochnig, P., R. Reuter, P. Bringmann, and R. Lührmann.** 1987. A monoclonal antibody against 2,2,7-trimethylguanosine that reacts with intact class U, small nuclear ribonucleoproteins as well as with 7-methylguanosine-capped RNAs. Eur. J. Biochem. **168**:461–467.
7. **Borer, R. A., C. F. Lehner, H. M. Eppenberger, and E. A. Nigg.** 1989. Major nucleolar proteins shuttle between nucleus and cytoplasm. Cell **56**:379–390.
8. **Carmo-Fonseca, M., R. Pepperkok, B. S. Sproat, W. Ansorge, M. S. Swanson, and A. I. Lamond.** 1991. *In vivo* detection of snRNP-rich organelles in the nuclei of mammalian cells. EMBO J. **10**:1863–1873.
9. **Carter, K. C., K. L. Taneja, and J. B. Lawrence.** 1991. Discrete nuclear domains of poly(A)RNA and their relationship to the functional organization of the nucleus. J. Cell Biol. **115**:1191–1202.
10. **Chang, D. D., and P. A. Sharp.** 1989. Regulation by HIV Rev depends upon recognition of splice sites. Cell **59**:789–795.
11. **Chida, K., and P. K. Vogt.** 1992. Nuclear translocation of viral Jun but not of cellular Jun is cell cycle dependent. Proc. Natl. Acad. Sci. USA **89**:4290–4294.
12. **Choi, Y. D., and G. Dreyfuss.** 1984. Monoclonal antibody characterization of the C proteins of heterogeneous nuclear ribonucleoprotein complexes in vertebrate cells. J. Cell Biol. **99**:1997–2004.
13. **Cochrane, A. W., K. S. Jones, S. Beidas, P. J. Dillon, A. M. Skalka, and C. A. Rosen.** 1991. Identification and characterization of intragenic sequences which repress human immunodeficiency virus structural gene expression. J. Virol. **65**:5305–5313.
14. **Cochrane, A. W., A. Perkins, and C. A. Rosen.** 1990. Identification of sequences important in the nucleolar localization of human immunodeficiency virus Rev: relevance of nucleolar localization to function. J. Virol. **64**:881–885.
15. **Cullen, B. R.** 1992. Mechanism of action of regulatory proteins encoded by complex retroviruses. Microbiol. Rev. **56**:375–394.
16. **Cullen, B. R., J. Hauber, K. Campbell, J. G. Sodroski, W. A. Haseltine, and C. A. Rosen.** 1988. Subcellular localization of the human immunodeficiency virus *trans*-acting *art* gene product. J. Virol. **62**:2498–2501.
17. **D'Agostino, D. M., B. K. Felber, J. E. Harrison, and G. N. Pavlakis.** 1992. The Rev protein of human immunodeficiency virus type 1 promotes polysomal association and translation of *gag/pol* and *vpu/env* mRNAs. Mol. Cell. Biol. **12**:1375–1386.
18. **Daly, T. J., K. S. Cook, G. S. Gray, T. E. Maione, and J. R. Rusche.** 1989. Specific binding of HIV-1 recombinant Rev protein to the Rev-responsive element *in vitro*. Nature (London) **342**:816–819.
19. **Davis, L., and G. Blobel.** 1986. Identification and characterization of a nuclear pore complex protein. Cell **45**:699–709.
20. **Dayton, A. I., E. F. Terwilliger, J. Potz, M. Kowalski, J. G. Sodroski, and W. A. Haseltine.** 1988. *Cis*-acting sequences responsive to the *rev* gene product of the human immunodeficiency virus.

J. Acquired Immune Defic. Syndr. **1**:441–452.
21. **Dayton, E. T., M. Douglas, D. Powell, and A. Dayton.** 1989. Functional analysis of CAR, the target sequence for the *rev* protein of HIV-1. Science **246**:1625–1629.
22. **Emerman, M., R. Vazeux, and K. Peden.** 1989. The *rev* gene product of the human immunodeficiency virus affects envelope-specific RNA localization. Cell **57**:1155–1165.
23. **Fakan, S., G. Leser, and T. E. Martin.** 1984. Ultrastructural distribution of nuclear ribonucleoproteins as visualized by immunocytochemistry on thin sections. J. Cell Biol. **98**:358–363.
24. **Fankhauser, C., E. Izaurralde, Y. Adachi, P. Wingfield, and U. K. Laemmli.** 1991. Specific complex of human immunodeficiency virus type 1 Rev and nucleolar B23 proteins: dissociation by the Rev response element. Mol. Cell. Biol. **11**:2567–2575.
25. **Feinberg, M. B., R. F. Jarret, A. Aldovini, R. C. Gallo, and F. Wong-Staal.** 1986. HTLV-III expression and production involve complex regulation at the levels of splicing and translation of viral RNA. Cell **46**:807–817.
26. **Felber, B. K., M. Hadzopoulou-Cladaras, C. Cladaras, T. Copeland, and G. N. Pavlakis.** 1989. *Rev* protein of human immunodeficiency virus type 1 affects the stability and transport of the viral mRNA. Proc. Natl. Acad. Sci. USA **86**:1495–1499.
27. **Freeman, J. W., A. Chatterjee, B. E. Ross, and H. J. Busch.** 1985. Epitope distribution and immunochemical characterization of nucleolar phosphoprotein C23 using ten monoclonal antibodies. Mol. Cell. Biochem. **68**:87–96.
28. **Fu, X.-D., and T. Maniatis.** 1990. Factor required for mammalian spliceosome assembly is localized to discrete regions in the nucleus. Nature (London) **343**:437–441.
29. **Green, M. R.** 1991. Biochemical mechanisms of constitutive and regulated pre-mRNA splicing. Annu. Rev. Cell Biol. **7**:559–599.
30. **Habets, W. J., M. H. Hoet, B. A. W. De Jong, A. Van der Kemp, and W. J. van Venrooij.** 1989. Mapping of B cell epitopes on small nuclear ribonucleoproteins that react with human autoantibodies as well as with experimentally induced mouse monoclonal antibodies. J. Immunol. **143**:2560–2566.
31. **Hammarskjöld, M.-L., J. Heimer, B. Hammarskjöld, I. Sangwan, L. Albert, and D. Rekosh.** 1989. Regulation of human immunodeficiency virus *env* expression by the *rev* gene product. J. Virol. **63**:1959–1966.
32. **Haseltine, W. A.** 1991. Regulation of HIV-1 replication, p. 1–42. *In* W. A. Haseltine and F. Wong-Staal (ed.), Genetic structure and regulation of HIV-1. Raven Press Ltd, New York.
33. **Heaphy, S., C. Dingwall, I. Ernberg, M. J. Gait, S. M. Green, J. Karn, A. D. Lowe, M. Singh, and M. A. Skinner.** 1990. HIV-1 regulator of virion expression (*rev*) protein binds to an RNA stem-loop structure located within the *rev* response element region. Cell **60**:685–693.
34. **Hope, T. J., D. McDonald, X. Huang, J. Low, and T. G. Parslow.** 1990. Mutational analysis of the human immunodeficiency virus type 1 *rev trans* activator: essential residues near the amino terminus. J. Virol. **64**:5360–5366.
35. **Huang, S., and D. L. Spector.** 1991. Nascent pre-mRNA transcripts are associated with nuclear regions enriched in splicing factors. Genes Dev. **5**:2288–2302.
36. **Huang, X., T. J. Hope, B. L. Bond, D. McDonald, K. Grahl, and T. G. Parslow.** 1991. Minimal *rev*-response element for type 1 human immunodeficiency virus. J. Virol. **65**:2131–2134.
37. **Jackson, D. A., A. B. Hassan, R. J. Errington, and P. R. Cook.** 1993. Visualization of focal sites of transcription within human nuclei. EMBO J. **12**:1059–1065.
38. **Jiménez-García, L. F., and D. L. Spector.** 1993. In vivo evidence that transcription and splicing are coordinated by a recruiting mechanism. Cell **73**:47–59.
39. **Kalland, K. H., E. Langhoff, H. J. Bos, H. Göttlinger, and W. A. Haseltine.** 1991. *Rex*-dependent nucleolar accumulation of HTLV-I mRNA. New Biol. **3**:389–397.
40. **Kiyokawa, T., M. Seiki, S. Iwashita, K. Imagawa, F. Shimizu, and M. Yoshida.** 1985. $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$, proteins encoded by the pX sequence of human T-cell leukemia virus type I. Proc. Natl. Acad. Sci. USA **82**:8359–8363.
41. **Kjems, J., M. Brown, D. D. Chang, and P. A. Sharp.** 1991. Structural analysis of the interaction between the human immunodeficiency virus *rev* protein and the *rev* responsive element. Proc. Natl. Acad. Sci. USA **88**:683–687.
42. **Kjems, J., and P. A. Sharp.** 1993. The basic domain of *rev* from human immunodeficiency virus type 1 specifically blocks the entry of U4/U6 · U5 small nuclear ribonucleoprotein in spliceosome assembly. J. Virol. **67**:4769–4776.
43. **Lamond, A. I., and M. Carmo-Fonseca.** The coiled body. Trends Cell Biol., in press.
44. **Lerner, E. A., M. R. Lerner, C. A. Janeway, and J. A. Steitz.** 1981. Monoclonal antibodies to nucleic acid-containing cellular constituents: probes for molecular biology and autoimmune disease. Proc. Natl. Acad. Sci. USA **78**:2737–2741.
45. **Lu, X., J. Heimer, D. Rekosh, and M.-L. Hammarskjöld.** 1990. U1 small nuclear RNA plays a direct role in the formation of *rev*-regulated human immunodeficiency virus *env* mRNA that remains unspliced. Proc. Natl. Acad. Sci. USA **87**:7598–7602.
46. **Maldarelli, F., M. A. Martin, and K. Strebel.** 1991. Identification of posttranscriptionally active inhibitory sequences in human immunodeficiency virus type 1 RNA: novel level of gene regulation. J. Virol. **65**:5732–5743.
47. **Malim, M. H., S. Böhnlein, J. Hauber, and B. R. Cullen.** 1989. Functional dissection of the HIV-1 *rev trans*-activator-derivation of a *trans*-dominant repressor of *rev* function. Cell **58**:205–214.
48. **Malim, M. H., R. Fenrick, D. W. Ballard, J. Hauber, E. Böhnlein, and B. R. Cullen.** 1989. Functional characterization of a complex protein-DNA-binding domain located within the human immunodeficiency virus type 1 long terminal repeat leader region. J. Virol. **63**:3213–3219.
49. **Malim, M. H., J. Hauber, R. Fenrick, and B. R. Cullen.** 1988. Immunodeficiency virus *rev trans*-activator modulates the expression of the viral regulatory genes. Nature (London) **335**:181–183.
50. **Malim, M. H., J. Hauber, S.-Y. Le, J. V. Maizel, and B. R. Cullen.** 1989. The HIV-1 *rev* transactivator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. Nature (London) **338**:254–257.
51. **Malim, M. H., D. F. McCarn, L. S. Tiley, and B. R. Cullen.** 1991. Mutational definition of the human immunodeficiency virus type 1 *rev* activation domain. J. Virol. **65**:4248–4254.
52. **Malim, M. H., L. S. Tiley, D. F. McCarn, J. R. Rusche, J. Hauber, and B. R. Cullen.** 1990. HIV-1 structural gene expression requires binding of the *rev trans*-activator to its RNA target sequence. Cell **60**:675–683.
53. **Mermer, B., B. K. Felber, M. Campbell, and G. N. Pavlakis.** 1990. Identification of *trans*-dominant HIV-1 *rev* protein mutants by direct transfer of bacterially produced proteins into human cells. Nucleic Acids Res. **18**:2037–2044.
54. **Ochs, R., M. Lischwe, P. O'Leary, and H. Busch.** 1983. Localization of nucleolar phosphoproteins B23 and C23 during mitosis. Exp. Cell Res. **146**:139–149.
55. **Olsen, H. S., A. W. Cochrane, P. J. Dillon, C. M. Nalin, and C. A. Rosen.** 1990. Interaction of the human immunodeficiency virus type 1 *rev* protein with a structured region in *env* mRNA is dependent on multimer formation mediated through a basic stretch of amino acids. Genes Dev. **4**:1357–1364.
56. **Olsen, H. S., P. Nelbock, A. W. Cochrane, and C. A. Rosen.** 1990. Secondary structure is the major determinant for interaction of HIV *rev* protein with RNA. Science **247**:845–848.
57. **Pines, J., and T. Hunter.** 1991. Human cell division: the involvement of cyclins A and B1, and multiple cdc2s, p. 449–463. *In* The cell cycle LVI. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
58. **Piñol-Roma, S., and G. Dreyfuss.** 1992. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature (London) **355**:730–732.
59. **Piñol-Roma, S., M. S. Swanson, J. G. Gall, and G. Dreyfuss.** 1989. A novel heterogeneous nuclear RNP protein with a unique distribution on nascent transcripts. J. Cell Biol. **109**:2575–2587.
60. **Raska, I., L. E. C. Andrade, R. L. Ochs, E. K. L. Chan, C.-M. Chang, G. Roos, and E. M. Tan.** 1991. Immunological and ultrastructural studies on the nuclear coiled body with autoimmune antibodies. Exp. Cell Res. **195**:27–37.
61. **Reuter, R., B. Appel, P. Bringmann, J. Rinke, and R. Lührmann.** 1984. 5′-terminal caps of snRNAs are reactive with antibodies

specific for 2,2,7-trimethylguanosine in whole cells and nuclear matrices. Exp. Cell Res. **154:**548–560.

62. **Riabowol, K. T., J. S. Fink, M. Z. Gilman, D. A. Walsh, R. H. Goodman, and J. R. Feramisco.** 1988. The catalytic subunit of cAMP-dependent protein kinase induces expression of genes containing cAMP-responsive enhancer elements. Nature (London) **336:**83–86.
63. **Richardson, W. D., A. D. Mill, S. M. Dilworth, R. A. Laskey, and C. Dingwall.** 1988. Nuclear protein migration involves two steps: rapid binding at the nuclear envelope followed by slower translocation through nuclear pores. Cell **52:**655–664.
64. **Rosen, C. A., E. Terwilliger, A. Dayton, J. Sodroski, and W. A. Haseltine.** 1988. Intragenic *cis*-acting *art*-gene-responsive sequences of the human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **85:**2071–2075.
65. **Sadaie, M. R., T. Benter, and F. Wong-Staal.** 1988. Site-directed mutagenesis of two *trans*-regulatory genes (*tat*-III, *trs*) of HIV-1. Science **239:**910–913.
66. **Saunders, W. S., C. A. Cooke, and W. C. Earnshaw.** 1991. Compartmentalization within the nucleus: discovery of a novel subnuclear region. J. Cell Biol. **115:**919–931.
67. **Schwartz, S., B. K. Felber, D. M. Benko, E. M. Fenyö, and G. N. Pavlakis.** 1990. Cloning and functional analysis of multiply spliced mRNA species of human immunodeficiency virus type 1. J. Virol. **64:**2519–2529.
68. **Schwartz, S., B. K. Felber, and G. N. Pavlakis.** 1992. Distinct RNA sequences in the *gag* region of human immunodeficiency virus type 1 decrease RNA stability and inhibit expression in the absence of Rev protein. J. Virol. **66:**150–159.
69. **Snow, C. M., A. Senior, and L. Gerace.** 1987. Monoclonal antibodies identify a group of nuclear pore complex glycoproteins. J. Cell Biol. **104:**1143–1156.
70. **Sodroski, J., W. C. Goh, C. Rosen, A. Dayton, E. Terwilliger, and W. A. Haseltine.** 1986. A second post-transcriptional activator gene required for HTLV-III replication. Nature (London) **321:** 412–417.
71. **Spector, D. L.** 1990. Higher order nuclear organization: three-dimensional distribution of small nuclear ribonucleoprotein particles. Proc. Natl. Acad. Sci. USA **87:**147–151.
72. **Spector, D. L., W. H. Schrier, and H. Busch.** 1983. Immunoelectron microscopic localization of snRNPs. Biol. Cell **49:**1–10.
73. **Terwilliger, E., R. Burghoff, R. Sia, J. Sodroski, W. A. Haseltine, and C. Rosen.** 1988. The *art* gene product of human immunodeficiency virus is required for replication. J. Virol. **62:**655–658.
74. **Venkatesh, L. K., and G. Chinnadurai.** 1990. Mutants in a conserved region near the carboxy-terminus of HIV-1 Rev identify functionally important residues and exhibit a dominant negative phenotype. Virology **178:**327–330.
75. **Wang, J., L.-G. Cao, Y.-L. Wang, and T. Pederson.** 1991. Localization of pre-messenger RNA at discrete nuclear sites. Proc. Natl. Acad. Sci. USA **88:**7391–7395.
76. **Xing, Y., C. V. Johnson, P. R. Dobner, and J. B. Lawrence.** 1993. Higher level organization of individual gene transcription and RNA splicing. Science **259:**1326–1330.
77. **Zamore, P. D., and M. R. Green.** 1991. Biochemical characterization of a U2snRNP auxiliary factor: an essential pre-mRNA splicing factor with a novel intranuclear localization. EMBO J. **10:**207–214.
78. **Zapp, M. L., and M. R. Green.** 1989. Sequence-specific RNA binding by the HIV-1 Rev protein. Nature (London) **342:**714–716.
79. **Zhang, M., P. D. Zamore, M. Carmo-Fonseca, A. I. Lamond, and M. R. Green.** 1992. Cloning and intracellular localization of the U2 small nuclear ribonucleoprotein auxiliary factor small subunit. Proc. Natl. Acad. Sci. USA **89:**8769–8773.