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A herpes simplex virus type 1 immediate-early gene product, IE63, regulates small nuclear ribonucleoprotein distribution

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ABSTRACT **Herpes simplex virus 1 (HSV-1), a nuclear replicating DNA virus, has 73 identified genes of which only 4 contain introns. For this reason the virus probably makes only minimal use of the cellular RNA-splicing machinery. Antigens associated with the small nuclear ribonucleoprotein particles (snRNPs) that are subunits of splicing complexes have been reported to redistribute in the nucleus and become concentrated into the intranuclear structures, the interchromatin granules, after HSV-1 infection [Martin, T. E., Barghusen, S. C., Leser, G. P. & Spear, P. G. (1987) *J. Cell Biol.* 105, 2069–2082]. We observe this snRNP redistribution upon HSV-1 infection, in which the widespread snRNP staining pattern changes to a restricted punctate distribution with a concomitant loss of coiled bodies in HSV-1-infected cells. We show here that expression of the immediate-early (IE) subset of HSV-1 genes is necessary and sufficient for snRNP redistribution. Using a series of HSV-1 mutants in different IE genes, we have established that specifically the product of the viral *IE63* (ICP27) gene is essential for this effect, and transfection experiments revealed that IE63 expression alone can cause the snRNP redistribution. Further, we show that the IE63 gene product colocalizes with the redistributed snRNP in the nucleus. The snRNP redistribution caused by HSV-1 infection resembles the effect seen after inhibition of transcription in uninfected cells. In HSV-1-infected cells, however, the snRNP redistribution is under the control of viral IE gene products and occurs during active virus gene transcription.**

In mammalian cells, the processes of splicing and polyadenylylation act on the primary transcript in the cell nucleus to generate mature mRNA. Nuclear pre-mRNA splicing requires a complex set of trans-acting splicing factors that bind to substrate RNAs in an ordered pathway to form an active splicing complex, or spliceosome. The major subunits of the spliceosome are the small nuclear ribonucleoprotein particles (snRNPs), specifically the U1, U2, U4/6, and U5 snRNPs (1). In addition, a number of non-snRNP protein-splicing factors are also required for splicing *in vitro* (for review, see ref. 2).

Herpes simplex virus type 1 (HSV-1) is a nuclear replicating virus and upon infection reduces host-cell gene expression and promotes expression of the viral genome. As HSV-1 infection proceeds, the levels of cellular RNA polymerase I and polymerase III activity are dramatically reduced, and host ribosomal RNA synthesis is shut down; there is also a reduction in the level of host mRNA transcription (3). An unusual and interesting feature of HSV-1 gene organization from the viewpoint of posttranscriptional processing is that only 4 of the 73 genes contain introns. Of the spliced genes, 3 are expressed from immediate-early (IE) times after infection, whereas the fourth one, *UL15*, is subsequently expressed at early-late times in infection. Transient expression of IE63 caused a reduction in the levels of spliced transcripts from a cotransfected reporter gene (4). If the virus inhibits cellular RNA splicing, the expression of host cellular genes would be profoundly affected, providing an advantage to the virus.

Indirect immunofluorescence studies have shown that antibodies specific for snRNP proteins label the nucleoplasm of mammalian cells in a characteristic widespread "speckled" pattern, excluding the nucleoli (5). This pattern includes a few densely staining foci that correspond to subnuclear organelles known as coiled bodies (for review, see ref. 6). Coiled bodies are evolutionarily conserved dynamic structures in the nuclei of both animal and plant cells, which exist as a concentration of snRNPs together with other phosphorylated proteins, such as coilin (7). The role of coiled bodies in snRNP function or the metabolism of nascent transcripts remains unknown. Upon HSV-1 infection, the snRNP distribution alters to form a pronounced punctate pattern (8); these punctate snRNP clusters correspond to clusters of interchromatin granules (8). HSV-1 infection has also been shown to affect distribution of the La antigen, a protein transiently associated with all RNA polymerase III transcripts. In this case, however, HSV-1 infection causes the La antigen to redistribute to the cytoplasm of the cell (9).

We show here that snRNP redistribution in HSV-1-infected cells is under the genetic control of the virus, and we have examined the HSV-1 genes necessary for this effect. Using a series of viral mutants and indirect immunofluorescence techniques, we show that viral IE gene products, and specifically the product of the *IE63* gene (also known as ICP27), are required for snRNP redistribution.

MATERIALS AND METHODS

Cell Culture. For indirect immunofluorescence, HeLa cells were grown on glass coverslips in Dulbecco's modified Eagle's medium (DMEM) (GIBCO) supplemented with 5% newborn calf serum and 5% fetal calf serum. Both Vero (African green monkey kidney) and BHK-C13 cells were grown on coverslips in DMEM supplemented with 10% fetal calf serum and 10% newborn calf serum, respectively.

Another cell line used, termed 2-2, is an IE63-complementing cell line derived from Vero cells (10). These cells were grown as for Vero cells, but the medium was supplemented with neomycin at a concentration of 750 μg/ml. Cells were used after reaching a confluency of 50–80%.

Treatment of Cells with Cycloheximide. Mock-infected cells were treated with cycloheximide at a concentration of 50 μg/ml and incubated at 37°C for 6 hr. For infected samples, 1 hr after cycloheximide treatment the virus was added to the cells at a multiplicity of infection of 10 plaque-forming units

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Abbreviations: HSV-1, herpes simplex virus 1; snRNP, small nuclear ribonucleoprotein particle; IE, immediate-early; ts, temperature-sensitive.

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per cell, and incubation was continued for a further 5–6 hr with cycloheximide.

Transfection Procedure. HeLa cells, grown on glass coverslips, were transfected with plasmid DNA using the lipofection method, as described by the manufacturer (Boehringer Mannheim). Each 50-mm Petri dish of cells was transfected with 10 μg of pSG130 (IE63) plasmid DNA and molar gene equivalent amounts of a plasmid encoding Vmw65 (VP16), a viral trans-activator of gene expression (10), and as transfection controls, Vmw65 alone and no DNA were used. The transfection was allowed to proceed for 18 hr; the cells were then washed and allowed to recover for a further 18 hr before the coverslips were harvested.

Antibodies. All antibodies were diluted in phosphate-buffered saline (PBS) before use. The U2 splicing snRNPs were labeled with the B″ monoclonal antibody 4G3 (11). The coiled-body antigen, p80 coilin, was detected by using a patient autoantiserum "sh" (12), at a dilution of 1:100. The HSV-1 major DNA-binding protein was detected with a monoclonal antibody, anti-UL29 (7381), at a dilution of 1:100, and the HSV-1 antigen IE110 was detected with an antiprotein antibody '96' raised in rabbits and used at a dilution of 1:100 (both provided by A. Cross, Institute of Virology, Glasgow). The HSV-1 IE63 antigen was detected with an antipeptide antibody raised in rabbits against the first 16 amino acids of the peptide, used at a 1:50 dilution (from H. Marsden, Institute of Virology, Glasgow).

Immunofluorescence. *Viruses.* Cells were infected with virus at a multiplicity of 10 plaque-forming units per cell in Dulbecco's modified minimal essential medium/1% serum. After incubation for 1.5–2 hr, the virus was removed, and fresh medium/1% serum was added. Cells were incubated for another 8–10 hr before coverslips were harvested and fixed. Slides were also fixed at 1-hr intervals over a 12-hr time course.

The virus strains used were wild-type HSV-1 strain 17; wild-type HSV-1 strain KOS (10); tsk, an HSV-1 mutant with a temperature-sensitive (ts) lesion in the *IE175* gene (13); 27-lacZ, an HSV-1 mutant virus (derived from wild-type KOS strain) carrying a lacZ cassette inserted into the 5′ untranslated leader sequence of *IE63*, which results in a nonfunctional IE63 gene product (10); A6b, an HSV-1 mutant virus with the entire *IE110* gene sequence deleted (14); δ325, an HSV-1 mutant with the entire *IE68* gene sequence deleted (15); and R3631, an HSV-1 mutant virus deleted for *IE12* (16).

Fixation. Fixation was done largely as described (17, 18). Briefly, cells on coverslips were washed twice with PBS and then fixed with 3.7% paraformaldehyde in CSK buffer (19) for 10 min, washed in PBS (for 5 min three times), and subsequently extracted with 0.5% (wt/vol) Triton X-100 in CSK buffer for 15 min at room temperature. Cells were then washed with PBS extensively.

Indirect immunofluorescence. Before being labeled, cells were briefly rinsed with 0.05% Tween 20/PBS. The cells were then treated with the primary antibody for 30 min at room temperature, washed three times in PBS for 5 min each time, and then treated with a fluorescein or Texas Red-labeled secondary antibody for 30 min at room temperature. For double-immunolabeling, the cells were consecutively incubated with one primary antibody, appropriate fluorochrome-conjugated secondary antibody, and then the other primary antibody and fluorochrome-conjugate.

All antibodies were diluted to an appropriate concentration with PBS. After being stained, the cells were washed extensively in PBS and mounted on slides with 67% glycerol/33% PBS.

Microscopy. Preparations were examined by using a Zeiss fluorescence microscope (IM35) with a ×100 objective. Confocal fluorescence microscopy was done by using the EMBL compact confocal microscope and the described settings (17).

RESULTS

Redistribution of snRNP Antigens in HSV-1-Infected Cells. HSV-1 infection has a dramatic effect on the normal distribution of cellular snRNPs (8). In mock-infected HeLa cells, a monoclonal antibody specific for the U2 snRNP B″ antigen shows a strong widespread speckled nucleoplasmic staining, which totally excludes staining of the nucleoli (Fig. 1*a*). A similar staining pattern was seen with the monoclonal antibody Y12, which recognizes the "Sm" proteins common to each of the major splicing snRNPs (data not shown). Mock-infected cells also exhibit a number a brightly staining foci when stained with an anti-U2 snRNP antibody, which by labeling with an anticoilin antibody have been identified as coiled bodies (Fig. 1*b*). However, by 6–8 hr after infection with wild-type HSV-1, the distribution of U2 snRNP changes to form a distinct punctate pattern (Fig. 1*c*). Later after infection (8–16 hr), these snRNP clusters become more pronounced and migrate to the periphery of the nucleus (data not shown). In agreement with Martin *et al.* (8), we find that the snRNP clusters colocalize with structures stained by a monoclonal antibody specific for interchromatin granules. We also find that the number of coiled bodies decreases (Fig. 1*d*), and the nucleolus disintegrates in HSV-1-infected cells (data not shown).

Viral Gene Expression Is Required for snRNP Redistribution. Upon HSV-1 infection, together with the viral DNA, viral capsid and tegument proteins enter the cell (16). Because the alteration of cellular snRNP distribution can be detected as early as 2 hr after infection, we wanted to determine whether viral gene expression was required for snRNP redistribution.

To address this question, cells were treated with cycloheximide, an inhibitor of mRNA translation, 1 hr before infection and then throughout the infection (Fig. 2*c*); as a control, mock-infected cells were treated identically for the same period (Fig. 2*a*). Results of this treatment show that even by 8 hr after infection, the snRNP redistribution effect was not detectable. We note that in both mock-infected and infected, cycloheximide-treated cells, the snRNP distribution has a more pronounced speckled appearance. This is

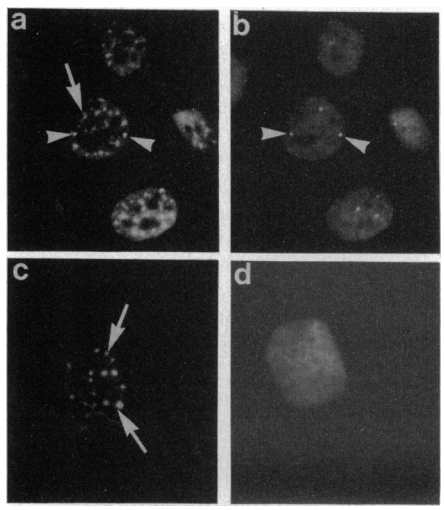

FIG. 1. Distribution of the B″, U2 snRNP antigen upon HSV-1 infection. Indirect immunofluorescence photomicrograph of mock-infected HeLa cells labeled with the anti-B″ U2 antibody (*a*) and anti-coilin antibody (*b*) and HeLa cells 8 hr after infection with wild-type HSV-1 labeled with the anti-B″ U2 snRNP antibody (*c*) and the anticoilin antibody (*d*). U2 labeling, which changed from a diffuse speckled pattern to a punctate distribution, is indicated by arrows; coiled bodies are indicated by arrowheads. Note that 8 hr after infection coiled bodies were no longer detectable. (×100.)

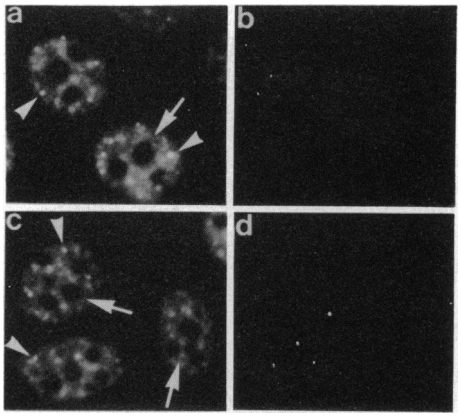

FIG. 2. Cycloheximide treatment inhibits snRNP redistribution. Indirect immunofluorescence photomicrographs of a cycloheximide-treated mock-infected HeLa cell labeled with the anti-B″ U2 antibody (*a*), a mock-infected HeLa cell labeled with the anti-IE63 antibody (*b*), a cycloheximide-treated HeLa cell 8 hr after infection labeled with anti-B″ U2 antibody (*c*), and a HeLa cell 8 hr after infection labeled with the anti-IE63 antibody (*d*). U2 B″ labeling is indicated with arrows; coiled bodies are indicated with arrowheads. (×100.)

probably from a stress-related phenomenon and not a virally induced effect. To ensure that the cycloheximide inhibition had been effective, the mock-infected and infected cells were also labeled with an antibody against the IE63 protein. Fig. 2 *b* and *d* indicate that no viral gene expression occurred. We conclude that the redistribution of the snRNPs requires viral gene expression.

Specific Viral Genes Are Essential for the snRNP Redistribution Effect. Several well-characterized HSV-1 mutants have been reported (10, 13–16). We performed a series of infections using such viral mutants to determine the gene(s) absolutely required for this snRNP redistribution effect (Figs. 3–5).

An infection was performed by using the HSV-1 mutant tsK. This mutant has a ts lesion in the *IE175* gene, (13) resulting in inhibition of early and late viral gene expression, together with the accumulation of IE gene products at the nonpermissive temperature (39.5°C). Fig. 3 *a* and *b* shows that at the permissive temperature (31°C), mutant tsK behaves as wild-type virus, causing snRNP redistribution. At the nonpermissive temperature, although only viral IE genes are expressed, snRNP redistribution is still clearly evident (Fig. 3 *d* and *e*). The mock-infected cells at 39.5°C show some snRNP clumping, presumably a stress-related phenomenon from a heat-related reduction in transcription levels. To demonstrate that nonpermissive conditions were attained, the cells were also labeled with an antibody against the major DNA-binding protein UL29, encoded by a gene expressed only at early-late times after infection. Fig. 3 *c* and *f* shows that UL29 was expressed at 31°C but was not expressed at 39.5°C.

Thus, viral IE gene expression alone is responsible for this snRNP redistribution effect, as no early or late gene products are synthesized under these conditions. There are five IE genes of HSV-1 (3), termed *IE175, IE110, IE68, IE63*, and *IE12*. Viruses with mutations in each of these genes were tested.

The HSV-1 IE63 Gene Product Causes the snRNP Redistribution Effect. Infection with mutant tsK demonstrated that snRNP redistribution was observed even when a nonfunctional IE175 product was expressed (Fig. 3). Our attentions turned to the remaining four HSV-1 IE genes. Hela cells and Vero cells were infected with viruses carrying deletions in the *IE110* (A6b), *IE68* (δ325), and *IE12* (R3631) genes, followed by indirect immunofluorescence. These experiments all

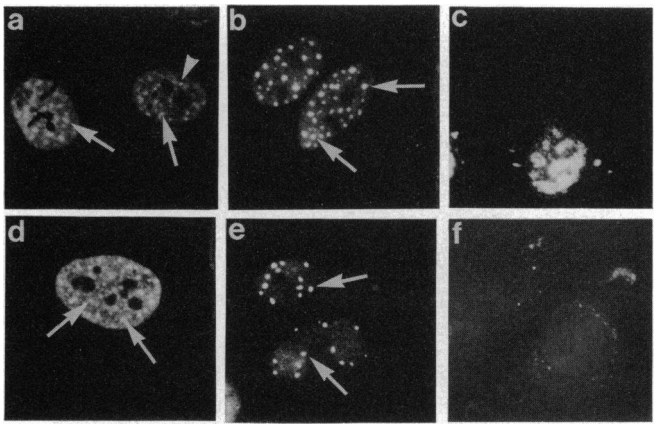

FIG. 3. Only viral IE gene expression is required for snRNP redistribution. Indirect immunofluorescence photomicrographs of mock-infected HeLa cells grown at 31°C for 14 hr and labeled with anti-B″ U2 snRNP antibody (*a*); HeLa cells 14 hr after infection with HSV-1 mutant tsK at the permissive temperature (31°C) labeled with anti-B″ U2 snRNP antibody (*b*), or 7381, an antibody against the viral protein UL29 (*c*). (*d*) Mock-infected HeLa cells grown at 39.5°C for 4 hr labeled with the anti-B″ U2 snRNP antibody. HeLa cells 4 hr after infection with mutant tsK at the nonpermissive temperature (39.5°C) labeled with the anti-B″ U2 snRNP antibody (*e*) or 7381, an antibody against viral protein UL29 (*f*). U2 labeling is indicated with arrows; coiled bodies are indicated with arrowheads. (×100.)

showed that the U2 snRNP redistributes into a highly punctate pattern in the absence of a functional representative of each of these genes (Fig. 4 *a–c*).

The viral IE gene product IE63, however, gave a different result. As IE63 is required for normal viral gene expression and viral replication, the mutant virus 27-lacZ must be propagated in a complementing cell line derived from Vero cells—namely, 2-2 cells (20). This stably transfected 2-2 cell line contains an IE63-encoding plasmid that must be trans-activated by the viral gene trans-activator VMW65 (a tegument protein) by either transfection or infection to promote expression of the IE63 gene product. Infection and subsequent indirect immunofluorescence was done in both the permissive and nonpermissive cell lines (Vero and 2-2 cells, respectively). Fig. 5 *a* and *c* illustrates that mock-infected Vero and 2-2 cells labeled with the anti-U2 B″ antibody both show the normal mock-infected-speckled snRNP staining pattern. Fig. 5*b* shows that the mutant virus 27-lacZ behaves as a wild-type virus in the complementing cell line. In Vero cells nonpermissive for 27-lacZ growth (Fig. 5*d*), no redistribution of the snRNPs was detected, and the U2 labeling pattern was as observed in mock-infected cells. As a control to show infection, the cells were routinely labeled with an antibody against the viral *IE110* gene. The cells were also

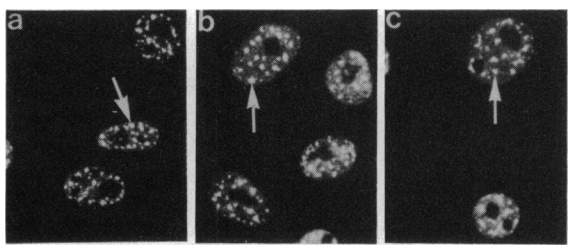

FIG. 4. Deletion of viral IE genes *IE110, IE68*, and *IE12* causes snRNP redistribution. Indirect immunofluorescence photomicrographs of HeLa cells labeled with the anti-B″ U2 snRNP antibody 8 hr after infection with HSV-1 virus deleted for *IE110 (a), IE68 (b)*, and *IE12 (c)*. The punctate U2 B″ labeling pattern is indicated with arrows. (×100.)

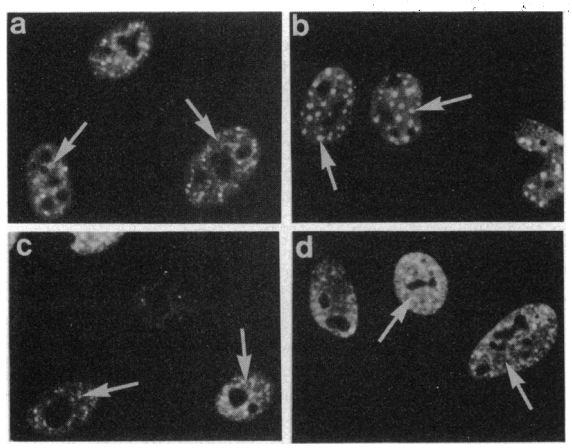

FIG. 5. IE63 is required for the snRNP redistribution effect. Indirect immunofluorescence photomicrographs of mock-infected 2-2 (*a*) and Vero cells (*c*) labeled with the anti-B″ U2 snRNP antibody and 2-2 cells (*b*) and Vero cells (*d*) 8 hr after infection with the IE63 mutant virus 27-lacZ, again labeled with anti-B″ U2 snRNP antibody. U2 B″ labeling is indicated with arrows. (×100.)

labeled with an antibody against IE63; there was no IE63 expression in 27-lacZ-infected Vero cells (data not shown).

Thus expression of the viral *IE63* gene is essential for the snRNP redistribution effect.

Expression of IE63 Alone Is Sufficient to Cause the snRNP Redistribution Effect. Transient transfection and subsequent immunofluorescence experiments have shown that HeLa cells expressing the IE63 gene product redistribute the U2 snRNP to a punctate pattern comparable with infected cells. Transfected HeLa cells were double-labeled with antibodies to IE63 and U2 snRNP. Fig. 6*a* is a photomicrograph of cells labeled with the IE63 antibody; the staining is distributed throughout the nucleus and cytoplasm. Cells expressing IE63 were also labeled with an antibody against the U2 snRNPs; Fig. 6*b* shows that those cells positive for IE63 expression also demonstrate a redistributed snRNP-labeling pattern, very similar to that seen in HSV-1-infected cells. All cells positive for IE63 expression exhibited this redistribution effect. The cells had been cotransfected with IE63 and Vmw65; control transfections with Vmw65 alone or with no DNA showed no snRNP redistribution effect.

IE63 Colocalizes with Redistributed snRNPs. Infected cells labeled with anti-IE63 antibody revealed that the IE63 gene product also formed a punctate distribution pattern (Fig. 7*b*). Control experiments demonstrate that mock-infected cells are not stained by the anti-IE63 antibody (data not shown). A double-labeling experiment shows that when these HSV-1-infected cells are stained by snRNP-specific antibodies, the same punctate pattern is seen (Fig. 7*a*). This colocalization of IE63 and snRNP-staining patterns indicates that the IE63 gene product is closely associated with splicing snRNPs in the nuclei of HSV-1-infected cells. As controls to prove the

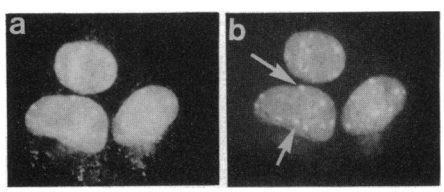

FIG. 6. Expression of IE63 alone can cause the snRNP redistribution effect. Indirect immunofluorescence photomicrograph of HeLa cells cotransfected with plasmids encoding IE63 and VMW65 labeled with an anti-IE63 antibody (*a*), and the same cells labeled with the anti-B″ antibody (*b*). The punctate U2 B″ labeling pattern in IE63-positive cells is indicated with arrows. (×100.)

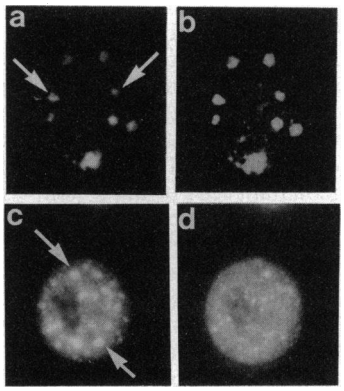

FIG. 7. Distribution of the B″, U2 snRNP antigen upon HSV-1 infection and colocalization with IE63. Indirect immunofluorescence photomicrograph of a HeLa cell 8 hr after infection with wild-type HSV-1 labeled with the anti-B″ U2 antibody (*a*) and the same cell labeled with an anti-IE63 antibody (*b*). A HeLa cell 4 hr after infection labeled with the anti-B″ U2 antibody (*c*) and the same cell labeled with the anti-IE110 antibody, '96' (*d*). Colocalization of IE63 with B″ is clearly demonstrated; the IE viral protein IE110 does not colocalize with the redistributed snRNP. The punctate U2 B″ labeling pattern is indicated by arrows. (×100.)

specificity of this colocalization, HeLa cells were also labeled with antibodies against the viral IE proteins IE110 and IE175. Fig. 7*d* demonstrates that although IE110 forms a spotted distribution pattern, it does not colocalize with the redistributed snRNPs (Fig. 7*c*); the IE175 protein also fails to colocalize with them (data not shown).

DISCUSSION

We have shown that the redistribution of splicing snRNPs within the nucleus that occurs after HSV-1 infection is under viral genetic control. In HSV-1-infected cells, the snRNP localization pattern changes from a widespread, speckled nucleoplasmic staining to one of dense snRNP clusters (8). This redistribution of snRNPs was shown here to depend upon HSV-1 IE gene expression, and specifically upon expression of the IE63 gene product. Inhibition of translation by cycloheximide before HSV-1 infection prevented snRNP redistribution. The redistribution is therefore not due to a nonspecific stress-related event or from the physical action of virus penetration into the cell. Nor is it the result of a viral capsid or tegument proteins, which include the viral host shut-off protein (3).

Time courses of infection from 0–3 hr revealed that the redistribution of snRNPs was detected after ≈1.5 hr, implicating viral genes expressed by 1–2 hr after infection in the redistribution effect. By analyzing both deletion and ts viral mutants, we have established that only one of the five HSV-1 IE genes, *IE63*, is essential for snRNP redistribution. A similar effect of *IE63* has been observed by R. M. Sandri-Goldin (personal communication). *IE63* is one of the five IE HSV-1 genes that are transcribed in the absence of *de novo* protein synthesis. In common with most HSV-1 transcripts, the IE63 pre-mRNA does not contain introns.

The IE63 protein is a trans-acting factor involved in the regulation of late gene expression (10, 21) and, in combination with other IE genes, can act as either a trans-repressor or trans-activator of gene expression (22). Our observation in this study that the IE63 protein colocalizes with the redistributed snRNP in HSV-1-infected cells provides additional evidence for some IE63 role in posttranscriptional processes. IE63 colocalized with snRNPs within the nucleus; these clusters of interchromatin granules migrated to the nuclear periphery as infection proceeded. Transfection experiments

determined that expression of the IE63 gene product alone was sufficient to cause snRNP redistribution, although IE63 could act in conjunction with other viral or cellular proteins to promote this effect under normal infection conditions.

Thus, HSV-1 encodes a factor that causes splicing snRNPs to change their intranuclear distribution, resulting in the accumulation of snRNPs in clusters of interchromatin granules and a concomitant loss of detectable coiled bodies. In uninfected cells, snRNPs appear in both coiled bodies and interchromatin granules (Fig. 1) (for review, see ref. 6). HSV-1 infection, therefore, dramatically changes the proportion of snRNPs in these distinct subnuclear compartments.

Interestingly, a similar alteration in snRNP distribution has been seen in uninfected cells after inhibition of transcription. In cells where transcription is blocked either by drug treatment (7) or as a result of terminal differentiation (M. Antoniou, M.C.-F., and A.I.L., unpublished data), snRNPs also leave coiled bodies and concentrate instead in clusters of interchromatin granules. This transcription-dependent snRNP redistribution effect in uninfected cells may be related to that seen in HSV-1-infected cells because in both cases the proportion of active snRNPs in the nucleus decreases. In the former case this is due to the absence of new transcripts, and in the latter case it is because the HSV-1 transcripts mostly lack introns.

HSV-1 is unusual in that only 4 of the 73 identified genes contain introns. Thus, a much smaller proportion of the splicing apparatus is probably active in HSV-1-infected cells. Interestingly, of the 4 genes that contain introns, 3 are expressed initially at IE times after infection. The other viral gene that requires splicing, *UL15*, is expressed from early-late times after infection. Therefore, at later times after infection the virus probably has minimal requirement for the cellular splicing machinery. The snRNP redistribution effect could be part of an active mechanism to inactivate snRNPs and, hence, to reduce the relative efficiency of host-cell gene expression. The effect may be necessary to facilitate the processing/transport of virus early and late mRNAs. Because most cellular pre-mRNAs must be spliced, inactivation of snRNPs would specifically inhibit expression of cellular and not inhibit expression of HSV-1 genes. Consistent with this hypothesis are recent findings that nuclear extracts from HSV-1-infected HeLa cells show reduced levels of pre-mRNA splicing activity *in vitro* (4). It is interesting in this regard that the cellular α-interferon proteins, which are major components of the host-cell antiviral response, are among the few cellular genes that lack introns. That the introns of HSV-1 are predominantly present in IE genes is consistent with the concept that the infectious strategy of HSV-1 includes a regulatory pathway for inactivating snRNPs. Future studies on the mechanism of IE63–snRNP interaction may, therefore, offer important insights regarding both the life cycle of HSV-1 and the *in vivo* organization and regulation of RNA processing in mammalian cells.

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