

## The gene product encoded by ORF 57 of herpesvirus saimiri regulates the redistribution of the splicing factor SC-35

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**The herpesvirus saimiri (HVS) gene product encoded by ORF 57 shares limited C-terminal similarity with herpes simplex virus 1 ICP27, a protein that has been demonstrated to be involved in the inhibition of host-cell splicing and is responsible for the redistribution of components of the spliceosome. It has previously been shown that ORF 57 can either activate or repress viral gene expression by a post-transcriptional mechanism. Furthermore, repression of gene expression by ORF 57 is dependent on the presence of an intron within the target gene coding region. In this report, it is shown that HVS infection results in the redistribution of the SC-35 splicing factor in the infected cell nucleus. Furthermore, the redistributed SC-35 colocalized with the ORF 57 protein product and expression of the protein alone was sufficient to cause the redistribution of the spliceosome components. These results suggest that the mechanism by which ORF 57 down-regulates expression of intron-containing genes involves the redistribution of the spliceosome complex.**

Herpesvirus saimiri (HVS) is a lymphotropic rhadinovirus ( $\gamma_2$  herpesvirus) of squirrel monkeys (*Saimiri sciureus*) that persistently infects its natural host without causing any obvious disease. However, HVS infection of other species of New World primates results in fulminant, polyclonal T-cell lymphomas and lymphoproliferative diseases (Fleckenstein & Desrosiers, 1982). HVS is also capable of transforming simian and human T lymphocytes to continuous growth *in vitro* (Beisinger *et al.*, 1992). Analysis of the genome of HVS (strain A11) indicates that it shares significant similarity with the herpesviruses Epstein–Barr virus (EBV), bovine herpesvirus-4, Kaposi's sarcoma-associated herpesvirus (human herpesvirus-

8) and murine gammaherpesvirus-68 (Albrecht *et al.*, 1992; Albrecht & Fleckenstein, 1990; Bublot *et al.*, 1992; Gompels *et al.*, 1988*a, b*; Neipel *et al.*, 1997; Russo *et al.*, 1996; Virgin *et al.*, 1997).

Gene expression during lytic replication of HVS is regulated by the products of two major transcription-regulating genes encoded by ORFs 50 and 57 (Whitehouse *et al.*, 1998*a*). ORF 50 produces two gene products, which are homologous to the EBV BRLF1 protein (Nicholas *et al.*, 1991) and function as sequence-specific transactivators (Whitehouse *et al.*, 1997). The ORF 57 protein is homologous to genes identified in all classes of herpesviruses, including the EBV transactivator encoded by BMLF1, ICP27 of herpes simplex virus (HSV), ORF 4 encoded by varicella-zoster virus and UL69 in human cytomegalovirus (Fleckenstein & Desrosiers, 1982; Kenney *et al.*, 1989; Nicholas *et al.*, 1988; Perera *et al.*, 1994; Winkler *et al.*, 1994).

The ORF 57 gene product has trans-regulatory functions; transactivation of late viral genes occurs independently of target gene promoter sequences and appears to be mediated at the post-transcriptional level, whereas repression of gene expression appears to correlate with the presence of introns, suggesting ORF 57 is functionally homologous to ICP27 (Whitehouse *et al.*, 1998*a, b*). ICP27 has been shown to contribute to the shut-off of host-cell protein synthesis and contributes to a decrease in cellular mRNA levels during infection (Hardwicke & Sandri-Goldin, 1994; Hardy & Sandri-Goldin, 1994; Hibbard & Sandri-Goldin, 1995). In addition, it has been shown that HSV-1 infection causes the redistribution of the small nuclear ribonucleoproteins (snRNPs), which are essential for the formation of the spliceosome complex (Martin *et al.*, 1987). The spliceosome removes introns from pre-mRNA and is a complex of many proteins, of which snRNPs are a major component (Green, 1991). Other non-snRNP proteins, such as spliceosome assembly factor SC-35, have been shown to be essential for spliceosome complex formation (Fu & Maniatis, 1990). It has been demonstrated that ICP27 is responsible for the redistribution of snRNPs (Phelan *et al.*, 1993), which is believed to be mediated through an interaction with its C terminus (Sandri-Goldin *et al.*, 1995; Sandri-Goldin & Hibbard, 1996).

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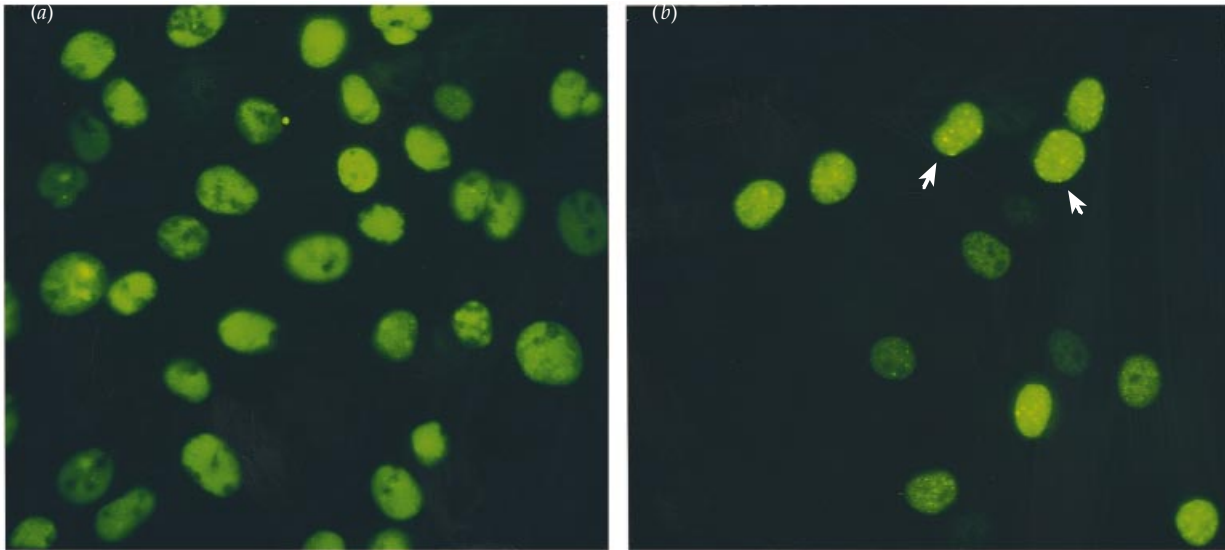


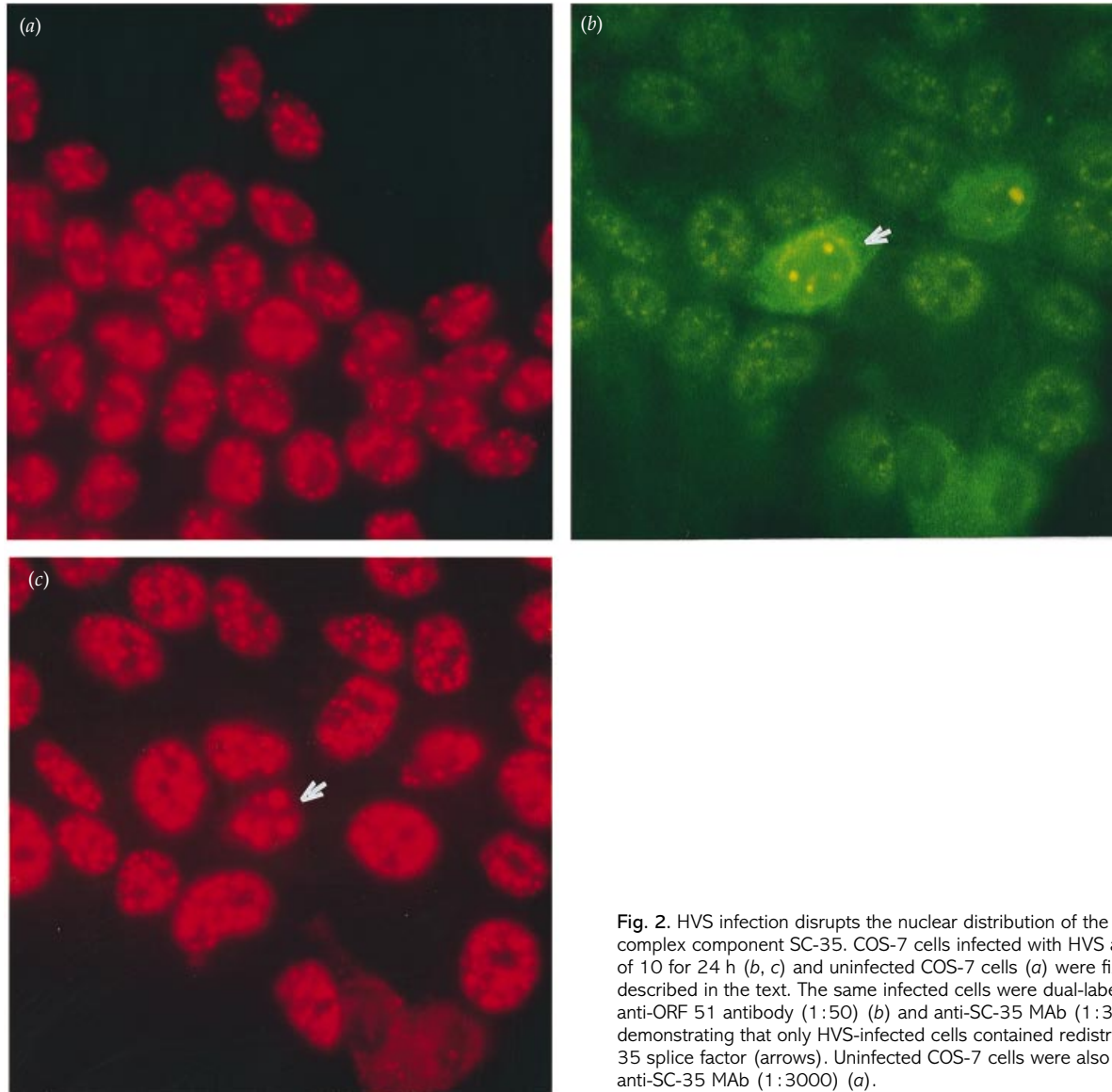
Fig. 1. Localization of the ORF 57 gene product in the nucleus of HVS-infected COS-7 cells. Cells were infected at an m.o.i. of 10 for 24 h and fixed as described in the text. Cells were probed with MAb SB and binding was detected with an anti-mouse FITC conjugate. A low dilution of SB showed a strong nuclear staining pattern (a), while a higher dilution (1:100) allowed a punctate staining pattern to be observed in the nucleus (b).

In this report, we demonstrate that HVS-infected cells display a reorganized distribution of the spliceosome component SC-35 and that this redistribution correlates with the presence of the ORF 57 gene product.

The sub-cellular localization of the ORF 57 gene product was investigated by using indirect immunofluorescence. This analysis was performed on COS-7 cells infected with HVS strain A-11. COS-7 cells were used because of their ease of culture and because they are fully permissive for HVS infection (Stevenson *et al.*, 1999). COS-7 cells were infected with HVS for 24 h at an m.o.i. of 10 before being fixed with 4% formaldehyde in PBS for 20 min at room temperature and then permeabilized in 0.5% Triton X-100 in PBS for 10 min at 4 °C. Cells were washed in PBS and blocked in 1% non-fat milk powder in PBS for 1 h at 37 °C. Cells were incubated with anti-ORF 57 monoclonal antibody (MAb) SB for 1 h at 37 °C before being washed in PBS and probed with an anti-mouse FITC conjugate (Dako) for 1 h at 37 °C. Cells were observed with a Zeiss Axiovert 135TV inverted microscope with a Neofluar  $\times 40$  oil-immersion lens. The SB MAb produced strong fluorescence in the nuclei of infected cells, as has been previously reported (Randall *et al.*, 1983; Whitehouse *et al.*, 1998*b*). However, more interestingly, serial dilutions of MAb SB resulted in a punctate staining pattern in the nuclei of infected cells, specifically with a 1:100 dilution of SB (Fig. 1).

The punctate staining pattern of MAb SB was similar to the staining pattern observed in HSV-1-infected cells (Martin *et al.*, 1987), which was later attributed to the effect of the IE gene ICP27 on the nuclear distribution of snRNPs (Phelan *et al.*, 1993). This suggested that components of the spliceosome might be redistributed in HVS-infected cells. In order to assess

the effect of HVS infection on the distribution of the components of the spliceosome, dual immunofluorescence analysis was performed. HVS-infected COS-7 cells were incubated with an antibody directed against the spliceosome factor SC-35, together with a rabbit antibody raised against the HVS structural protein encoded by ORF 51, which allowed the identification of infected cells. ORF 51 encodes a putative viral glycoprotein and is first detectable at 24 h post-infection (P. Gibson, personal communication). COS-7 cell coverslip cultures were infected and subsequently fixed in the manner described above. Cells were incubated with an anti-SC-35 MAb (Sigma) for 1 h at 37 °C. Mouse-antibody labelling was detected by incubation for 1 h at 37 °C with an anti-mouse Texas red conjugate (Vector). The anti-ORF 51 antibody was then overlaid onto the cells for 1 h at 37 °C and labelling was detected with an anti-rabbit FITC conjugate for 1 h at 37 °C. It was observed that the anti-SC-35 MAb produced a characteristic, evenly distributed speckled pattern in the nuclei of uninfected cells (Fu & Maniatis, 1990; Martin *et al.*, 1987), while the ORF 51 antibody was unreactive in uninfected cells. However, in COS-7 cells infected with HVS, which were identified by the positive fluorescent signal of the anti-ORF 51 antibody, the SC-35 MAb demonstrated a distinct punctate staining pattern (Fig. 2). The punctate staining of the SC-35 MAb in the infected cell nuclei had a similar pattern to that of the anti-ORF 57 MAb and appeared to localize to the same distinct nuclear domains. However, due to the intense fluorescence of the SC-35–Texas red signal, a slight punctate staining is observed in uninfected cells resulting from bleed-through when viewed through the FITC channel. This effect was observed in subsequent colocalization experiments. A



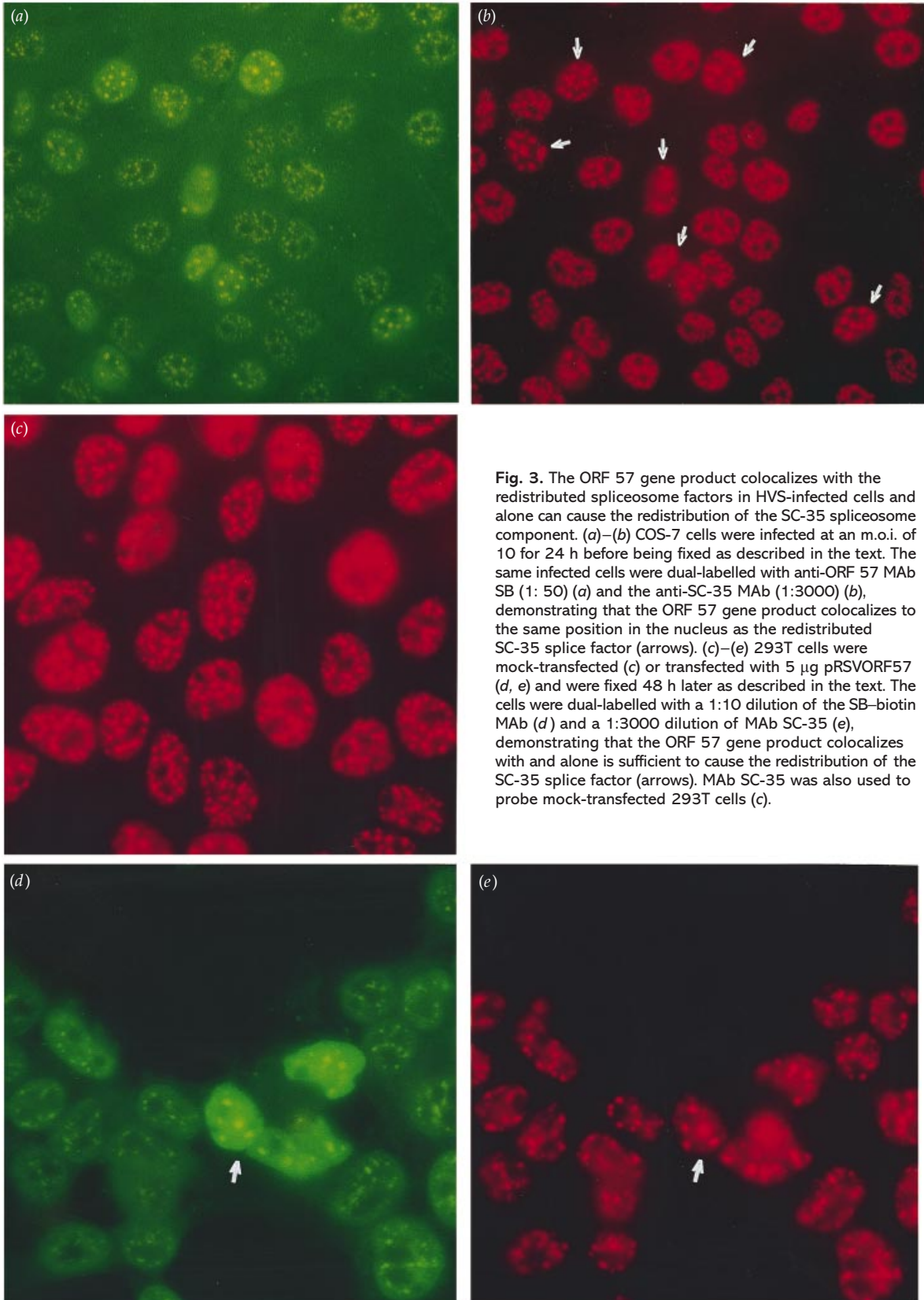
**Fig. 2.** HVS infection disrupts the nuclear distribution of the spliceosome complex component SC-35. COS-7 cells infected with HVS at an m.o.i. of 10 for 24 h (b, c) and uninfected COS-7 cells (a) were fixed as described in the text. The same infected cells were dual-labelled with anti-ORF 51 antibody (1:50) (b) and anti-SC-35 MAb (1:3000) (c), demonstrating that only HVS-infected cells contained redistributed SC-35 splice factor (arrows). Uninfected COS-7 cells were also labelled by anti-SC-35 MAb (1:3000) (a).

similar redistribution was also observed when using an antibody directed against the U2 snRNP (Euro-Diagnostica) and has also been observed in OMK cells when using both anti-SC-35 and anti-U2 snRNP antibodies (results not shown).

The ORF 57 gene product and the spliceosome component SC-35 showed similar patterns of staining within nuclei of HVS-infected cells. To investigate whether the spliceosome components were redistributed to the same region of the nucleus as the ORF 57 gene product, dual labelling was performed with the anti-ORF 57 MAb SB and the anti-spliceosome MAb SC-35. However, as both MAbs were of the same IgG class, the anti-ORF 57 (SB) MAb was first labelled with biotin (Boehringer), following the manufacturer's instructions, allowing SB binding to be detected with an anti-biotin FITC conjugate (Sigma). COS-7 cells were infected and fixed in the manner described above and incubated first with MAb SC-

35 for 1 h at 37 °C. SC-35 antibody binding was detected by incubation with an anti-mouse Texas red conjugate for 1 h at 37 °C. The coverslips were then incubated for 1 h at 37 °C with the SB-biotin complex and SB-biotin binding was detected by probing with an anti-biotin FITC conjugate for 1 h at 37 °C. It was observed that the SC-35 staining patterns in HVS-infected cells were identical to the staining pattern of the SB-biotin MAb observed in the same cell (Fig. 3a, b). Only cells that were labelled by SB-biotin had the redistributed spliceosome component staining pattern. Moreover, an identical result was also observed with the anti-U2 snRNP MAb (result not shown).

Transient expression of the ORF 57 gene product alone was sufficient to cause the redistribution of the spliceosome components when 293T cells were transfected with the eukaryotic expression construct pRSVORF57 (Whitehouse *et*



**Fig. 3.** The ORF 57 gene product colocalizes with the redistributed spliceosome factors in HVS-infected cells and alone can cause the redistribution of the SC-35 spliceosome component. (a)–(b) COS-7 cells were infected at an m.o.i. of 10 for 24 h before being fixed as described in the text. The same infected cells were dual-labelled with anti-ORF 57 MAb SB (1: 50) (a) and the anti-SC-35 MAb (1:3000) (b), demonstrating that the ORF 57 gene product colocalizes to the same position in the nucleus as the redistributed SC-35 splice factor (arrows). (c)–(e) 293T cells were mock-transfected (c) or transfected with 5 µg pRSVORF57 (d, e) and were fixed 48 h later as described in the text. The cells were dual-labelled with a 1:10 dilution of the SB-biotin MAb (d) and a 1:3000 dilution of MAb SC-35 (e), demonstrating that the ORF 57 gene product colocalizes with and alone is sufficient to cause the redistribution of the SC-35 splice factor (arrows). MAb SC-35 was also used to probe mock-transfected 293T cells (c).

*al.*, 1998*b*). 293T cells were used because they gave a higher transfection efficiency when compared with COS-7 cells. 293T cells grown on coverslips were transfected with 5 µg pRSVORF57 by using Lipofectamine (Gibco BRL) according to the manufacturer's instructions. Cells were incubated for 48 h before being fixed as described above. The cells were dual-labelled in the manner described above, first with MAb SC-35 followed by an incubation with an anti-mouse Texas red conjugate. The cells were then incubated with the SB-biotin MAb before being washed in PBS and SB-biotin binding was detected by probing with an anti-biotin FITC conjugate. A staining pattern identical to that seen in the dual-labelling experiments with SC-35 and SB-biotin in HVS-infected cells was observed in cells transfected with pRSVORF57 (Fig. 3 *c-e*). Only in cells positive for the ORF 57 gene product was there a redistribution of the SC-35 spliceosome component; mock-transfected cells showed no redistribution of spliceosome components. Once again the anti-U2 snRNP MAb gave a result identical to that obtained with the anti-SC-35 MAb (result not shown).

In this report we have demonstrated that the ORF 57 gene product is responsible for the redistribution of the spliceosome complex during HVS infection. These results give some indication of how the ORF 57 gene product may modulate the post-transcriptional processing of mRNA, as described in our previous reports (Whitehouse *et al.*, 1998*a, b*). It would seem likely that the redistribution of the spliceosome complex has a profound effect upon the splicing capability of a cell and consequently on the processing of viral intron-containing mRNA. Therefore, we believe the role of the ORF 57 gene product in the redistribution of the spliceosome components may be similar to that of HSV-1 IE gene product ICP27, which has been shown to redistribute the snRNP components of the spliceosome (Phelan *et al.*, 1993; Sandri-Goldin *et al.*, 1995). In addition, similar results have recently been observed with the EBV Mta protein, a homologue of ORF 57 (Semmes *et al.*, 1998).

The mechanism of ORF 57 interaction with the spliceosome components is as yet unknown, although if comparisons are made with ICP27, it could involve the C-terminal region of the protein. The C-terminal portion of ICP27 has been shown to contain a repressor region that is essential for the inhibition of splicing. This region is also required for the redistribution of the snRNPs, although a temperature-sensitive mutant in the repressor region allowed the redistribution of the snRNPs but did not inhibit host-cell splicing (Sandri-Goldin *et al.*, 1995). There is a limited degree of similarity between the C-terminal regions of ORF 57 and ICP27 (Brown *et al.*, 1995). Interestingly, this region of ICP27 contains a 'zinc knuckle' motif that is also found in a number of splicing factors (Sandri-Goldin & Hibbard, 1996). It is possible that ORF 57 binds directly to the snRNA via its 'zinc knuckle', although a direct protein-protein interaction between the spliceosome component and the ORF 57 gene product may also be possible.

In summary, we have demonstrated that HVS infection results in the redistribution of the SC-35 and snRNP U2 components of the spliceosome. We have also shown that the redistributed spliceosome components colocalize to regions of the nucleus where the ORF 57 gene product is concentrated. Furthermore, the expression of ORF 57 alone is sufficient to cause the redistribution of the spliceosome components.

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