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Structural and Functional Characterization of Herpes Simplex Virus 1 Immediate-Early Protein Infected-Cell Protein 22

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Of the five HSV1 immediate-early proteins, infected-cell protein 22 (ICP22), the product of the Us1 gene, is a member whose function is less understood. In order to promote better understanding of the role of ICP22 in viral replication, mutation and fluorescence techniques were used to investigate the biochemical relationship between ICP22's structure and nuclear localization, and the CAT assay was used to analyze the relationship between ICP22's structure and its transcriptional repression. The results of these experiments implied (i) ICP22 is localized to small dense nuclear bodies and is paired with the SC-35 domain in the nucleus, (ii) ICP22 localization in a punctate state requires completion of the main sequence which includes the 1–320th amino acids, (iii) a conservative mutation in the nucleotidylylation site is important for its nuclear localization and transcriptional repression, and (4) despite possessing the same amino acid sequence as the ICP22 carboxyl-terminal, Us1.5 was distinct from ICP22 in location and function.

Key words: gene expression regulation, human herpes virus 1, ICP22 protein, nucleotidylylation, transcription.

Abbreviations: CAT, chloramphenicol acetyl transferase; CHO, Chinese hamster ovary; DMEM, Dulbecco's modified Eagle's medium; HSV1, herpes simplex virus 1; ICP22, infected cell protein 22; IE, immediate early; GFP, green fluorescence protein.

Herpes simplex virus 1 (HSV1) is a large DNA virus with a complicated, multilevel mechanism of transcriptional regulation; it has more than 80 open reading frames to express various functional and structural proteins during infection (*1*). However, more than half of these proteins are dispensable for viral replication, at least in some cultured cell lines (*2*). Given the fact that HSV1 possesses different infectious characteristics in different cells, such as epithelial cells and neurons (*3*), some of these proteins are likely to play specific roles in particular cellular environments. Biochemical analysis of these functional proteins will provide more detailed information about the mechanism for HSV1 induction of lytic and latent infection in different cells.

Of the five HSV1 immediate-early (IE) proteins, infected-cell protein 22 (ICP22), the product of the α22 gene, is a member whose function is less understood than those of the other four IE proteins (*1*). Some studies have provided evidence that this protein is dispensable for viral replication in certain cell lines (*4–6*). However, others have suggested that it plays an important role in the regulation of viral gene expression (*6*, *7*). These seemingly conflicting data point to a need for further investigation of ICP22's role in viral infection through structural and functional analyses.

ICP22 is one of two proteins encoded by the α22 gene, US1.5 being the other; there are independent promoters for each protein. ICP22 is composed of 420 amino acids with alternating acidic and basic domains. US1.5 is composed of a 274-amino-acid sequence of ICP22, beginning at Met147 (*8*, *9*). These two semihomologous proteins are likely to have at least one common role in viral replication. Some functional-anatomic data suggest that ICP22 has two sets of functions, one linked to its unique N-terminal sequence and the other linked to the sequence it shares with US1.5 (*10*). These two sets of functions, which have not yet been fully and clearly described, include nucleotidylylation at the specific site of the 76–79th amino acid residues (Arg-Arg-Ala-Pro), interaction with cellular proteins, and regulation of viral gene expression (*11–15*).

With respect to ICP22's role in viral replication, data indicate that ICP22 is involved in the posttranslational modification of RNA polymerase II that is reflected in an "intermediate" electrophoretic mobility between those of the hyperphosphorylated (RNA POL IIo) and hypophosphorylated (RNA POL IIa) states (*7*, *16*, *17*). This action occurs following phosphorylation of ICP22 by a protein kinase encoded by UL13 and Us3, and involves a physical interaction with Cdk9, a constitutively active cyclin-dependent kinase linked to transcriptional regulation. On the other hand, Prod'hon reported the results of a chloramphenicol acetyl-transferase (CAT) assay that suggest ICP22 functions as a repressor of transcription initiated by the IE gene promoter and imply that ICP22 is capable of negatively regulating the expression of viral α-genes (*13*). Previous data obtained in our laboratory also constitute evidence of this effect (Cun, W. and Li, Q., unpublished data).

In light of these findings, ICP22 may be inferred to have a multifunctional role in viral replication. It has been repeatedly shown that ICP22 is specifically localized to the nucleus in small dense nuclear bodies (*18*, *19*). During

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late viral infection, ICP22 was found to be colocalized in small dense nuclear bodies with UL4, a late viral protein of unknown function (*20*). Although cellular biological analysis has not clearly elucidated the function of small dense nuclear bodies, some work suggests that these nuclear organelles are involved in transcriptional regulation (*7*, *13*). This raises the possibility of a link between the specific localization of ICP22 and its biological function in viral infection, as well as a connection between its specific localization and its structure.

The work described in this paper sought to address these questions in order to promote better understanding of the role of ICP22 in viral replication. First, we used mutation and fluorescence techniques to investigate the biochemical relationship between ICP22's structure and nuclear localization. Second, we used the CAT assay to analyze the relationship between ICP22's structure and its transcriptional repression, which was demonstrated in our previous work (Cun, W. and Li, Q., unpublished data). The results demonstrate a relationship between the structural domains of ICP22, and both its nuclear localization and transcriptional repression.

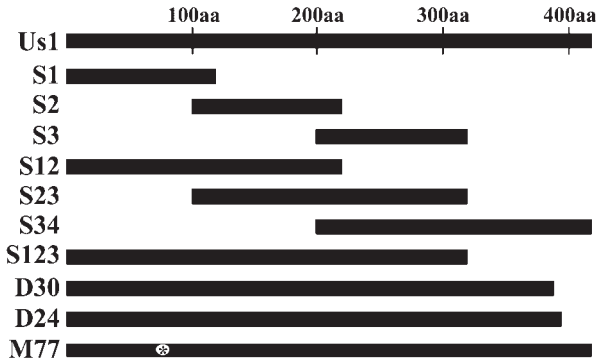

Fig. 1. **Schematic diagram of various Us1-derived sequences which were used to construct different ICP22 mutations.** These deletion mutations, S1 (1–360 nt), S2 (300–660 nt), S3 (600–960 nt), S12 (1–660 nt), S23 (300–960 nt), S34 (600–1260 nt), S123 (1–960 nt), D30 (1–1170 nt), and D24 (1–1188 nt), and the conservative mutation M77 (*229–234 nt: AGGGCC→AAGCTT) were inserted into eukaryotic plasmid pEGFPN2 or pcDNA3, respectively.

MATERIALS AND METHODS

Cells and Virus—Vero cells (maintained in this laboratory) were grown in DMEM containing 10% fetal calf serum under 5% CO_2 at 37°C to form a monolayer in the flask. Chinese hamster ovary (CHO) cells were grown in complete Ham's F12 media containing 5% fetal calf serum under 5% CO_2 at 37°C to a confluence of 80% in a 50 mm plate. Herpes simplex virus 1 (obtained from the Institute of Virology, Beijing) was grown in Vero cells.

Plasmid Construction—A plasmid series expressing fusion proteins of fluorescence protein and different ICP22 mutants was constructed using the deletion strategy outlined in Fig. 1. For this strategy, pEGFPN2 (Invitrogen) containing the green fluorescence protein gene was used as a eukaryotic vector. The primers used to obtain the sequences of the different HSV1 α22 gene mutant fragments were as follows:

p1: AGCAGATCTGGATGGCCGACATTTCCCCAG;
p2: AGCAAGCTTGGGGGAATGTCGTCATAAG;
p3: AGCAGATCTGGATGGATGCGTCGGTGTCGGAC;
p4: AGCAAGCTTAGGTGGCGCAGGCGGTTGG;
p5: AGCAAGCTTGATGCTGTTTCGGGTCCTG;
p6: AGCGAATTCGAGCGTGTGGTCCG;
p7: AGCGAATTCGGCGCGACCCGCCCCAG;
p8: AGCGAATTCACACTTGCGGTCTTCTG;
p9: AGCGAATTCCGGCCGGAGAAACGTGTCGC.

Primers p1 and p9 were used to construct pEGFPN-Us1, containing the full encoding sequence of ICP22. Primers p1 and p2 were used to construct pEGFPN-S1, containing amino acids 1–120 of the ICP22-encoding sequence. Primers p3 and p4 were used to construct pEGFPN-S2, containing amino acids 100–220 of the ICP22-encoding sequence. Primers p5 and p6 were used to construct pEGFPN-S3, containing amino acids 200–320 of the ICP22-encoding sequence. Primers p1 and p4 were used to construct pEGFPN-S12, containing amino acids 1–220 of the ICP22-encoding sequence. Primers p3 and p6 were used to construct pEGFPN-S23, containing amino acids 100–320 of the ICP22-encoding sequence. Primers p5 and p9 were used to construct pEGFPN-S34, containing amino acids 200–420 of the ICP22-encoding sequence. Primers p1 and p6 were used to construct pEGFPN-S123, containing amino acids 1–320 of the ICP22-encoding sequence. Primers p1 and p7 were used to construct pEGFPN-D30, containing amino acids 1–390 of the ICP22-encoding sequence. Primers p1 and p8 were used to construct pEGFPN-D24 containing amino acids 1–396 of the ICP22-encoding sequence.

A plasmid series expressing different ICP22 mutations was constructed with pcDNA3 (Invitrogene) and the mutant fragments described above. The primers used in this process had the same sequence with a *Bam*HI or *Eco*RI site and terminate code TGA added in reverse-primers. Eukaryotic expression vectors pcDNA-Us1, pcDNA-S1, pcDNA-S2, pcDNA-S3, pcDNA-S12, pcDNA-S23, pcDNA-S34, pcDNA-S123, pcDNA-D30, and pcDNA-D24 were constructed using the series of primers described above. Two additional plasmids, pEGFPN-M77 and pcDNA-M77, which contained the entire ICP22-encoding sequence and had a conservative substitution of $Arg^{77}Ala^{78}$→$Lys^{77}Leu^{78}$, were constructed according to the standard point mutation protocol (*21*), and using pEGFPN-Us1, pcDNA-Us1, and specific primers p10 (AGCAAGCTTACGGCCACCGATG) and p11 (AGCAAGCTTCCCCGGAGGCTTG), respectively. pEGFPN-Us1.5 and pcDNA-Us1.5 were constructed with pEGFPN2, pcDNA3 and the gene fragment encoding Us1.5. pCAT-332 was constructed with pCAT3-Basic (Promega) and the upstream sequence of the HSV1 α4 gene in previous work (Cun, W. and Li, Q., unpublished data).

Cell Transfection—All plasmids were linearized by digestion with restriction enzymes. The CHO cells were grown in 6-well plates to 70% confluence, and then transfected with Lipofectamine 2000 and 1 μg/well of the required plasmid according to the manufacturer's instructions. The transfection control comprised linearized pcDNA3. Each transfection experiment was repeated

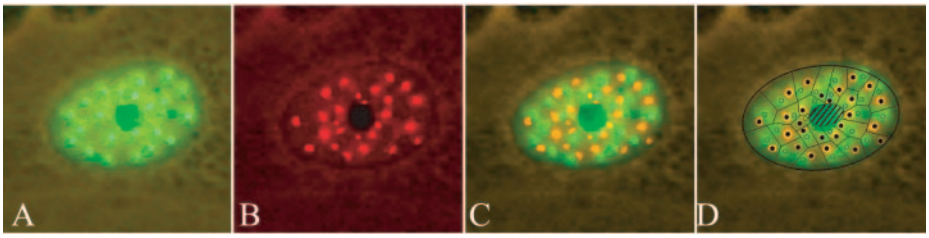

Fig. 2. **ICP22 is localized to small dense nuclear bodies and is paired with the SC-35 domain.** pEGFPN-Us1 was transfected into CHO cells in conjunction with immunofluorescence staining using an antibody to SC-35. ICP22-GFP was localized to small green dense bodies (A) and SC-35 mAb gave many red spots (B) throughout the nucleoplasm of the same transfected CHO-k1 cell with pEGFPN2-ICP22, and the two photographs were then overlaid (C). A schematic diagram (D) shows the ICP22 dense bodies (open circles) paired with the SC-35 domain (solid circles) outside the nucleolus (hatched area).

2–3 times, and each transfection sample was added to three wells of cells at the same time.

Fluorescence Detection—Transfected CHO cells were grown overnight on slides and then fixed in 2% formaldehyde/PBS/0.2% Triton X-100 for 10 min at 4°C. The fixed cells were observed under a fluorescence microscope (Nikon ECLIPSE E600) for protein localization or submitted to indirect immunofluorescence detection. The fixed cells were permeabilized for 5 min in cold acetone (–20°C) and then blocked with 20% bovine serum albumin/PBS/0.5% Tween-20 for 30 minutes at room temperature. Purified mouse anti-SC35 monoclonal antibodies (BD Pharmingen, Cat# 556363) were applied to the slides, and then the slides were incubated for 2 h at 37°C. The slides were washed for 10 min in PBS, and then treated for 1 h with secondary rhodamine–conjugated goat anti–mouse immunoglobulin G1 antibodies (Santa Cruz Biotechnology, Cat# sc-2084), washed three times in PBS, and mounted. Observation was performed under a fluorescence (Nikon ECLIPSE E600) or confocal microscope (ZEISS LSM 510META).

Western Blot—The transfected CHO cells were grown in complete medium for 36 h, scraped off with a rubber policeman, and then washed three times with PBS. The washed cells were lysed in RSB buffer for 1 h at 4°C. The supernatant was collected by centrifugation at 11,000 × *g* and 4°C for 10 min and separated by 12% SDS-PAGE. Following this step, the target protein was transferred to a NC membrane and then analyzed with specific antiserum against the ICP22 N-terminal fragment of 36 amino acids expressed in *E. coli* using the standard Western blot protocol (*22*).

CAT Assay—After being maintained in Ham's F12 medium containing 5% fetal calf serum for 36 h, the transfected CHO cells were washed with PBS, lysed with 1× Reporter Lysis Buffer (Promega), scraped off, and then transferred to a microcentrifuge tube. A supernatant was obtained by centrifugation at 11,000 × *g* for 5 min. The CAT and β-galactosidase enzyme assays were performed for the same cell extract. Some of the supernatant was incubated at 60°C for 10 min, and the CAT assay system was composed of an extract of transfected cells (10 μl), [3,5-^{3}H]chloramphenicol (0.25 μCi, New England Laboratory), *n*-butyryl-CoA (5 μl, 5 mg/ml), and Tris-HCl (100 μl, pH 8.5, 0.25 M), in a total reaction volume of 125 μl. This reaction system was incubated at 37°C for 1–5 h, shaken strongly for 30 s in a Vortex with 300 μl of mixed xylenes, and then spun at 11,000 × *g* to separate the two phases. The upper-phase xylenes were collected and added to 100 μl of 0.25 M Tris-HCl for one additional extraction. After second collection, the

Table 1. The numbers of punctate ICP22 and SC-35 in transfected cells.

Field	The number of ICP22 dense bodies	The number of the SC35 domain
I	29	29
	61	58
	44	42
II	17	18
	14	14
	10	11
	8	8
III	41	36
	19	19
	47	32
	20	19
IV	36	31
	15	15
V	21	21
	27	27
	29	28
	49	46
VI	7	7
	11	11
VII	35	32
	47	45
	32	31
VIII	26	24
	22	22
	34	30
	10	11
	13	13
IX	25	23
	46	43
	25	19
	26	26
X	14	14
	15	13
	17	17

[a] $r = 0$

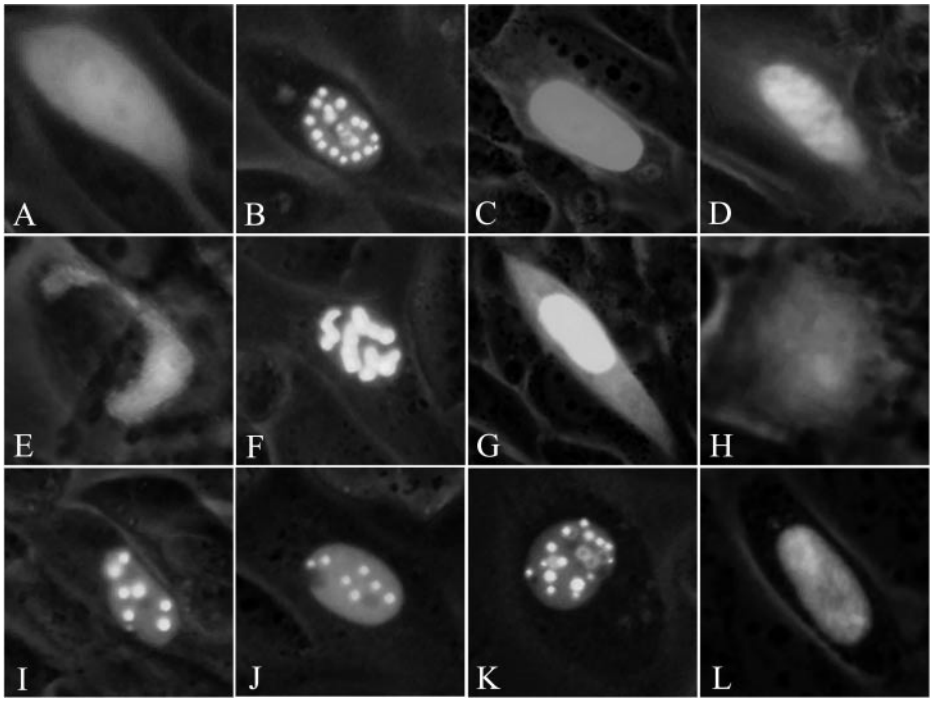

Fig. 3. **Localization of ICP22–GFP hybrids in living cells.** CHO cells were transiently transfected with constructs expressing the fusion proteins and then analyzed 36 h posttransfection by fluorescence microscopy without fixation. A control experiment demonstrated cytoplasmic and nuclear localization of GFP alone (A); expression of full-length ICP22–GFP resulted in nuclear localization which exhibited a typical speckle pattern (B); expression of various ICP22 mutant–GFP hybrids (C: S1-GFP, D: S2-GFP, E: S3-GFP, F: S12-GFP, G: S23-GFP, H: S34-GFP, I: S123-GFP, J: D30-GFP, K: D24-GFP, and L: M77-GFP) revealed ICP22 localization in a punctate state within small dense nuclear bodies requires completion of the N-terminal sequence.

upper-phase xylenes was put into a scintillation counter with 800 μl of scintillation fluid, and a standard curve of CAT activity was obtained using serial CAT enzyme (Promega) dilutions of 0.1 to 0.003125 unit. One unit (U) was defined as the amount of enzyme required to transfer 1 nmol of acetate to chloramphenicol in one minute at 37°C. To normalize for transfection efficiency, the CAT expression level was normalized as to the level of β-galactosidase expression. Galactosidase activity was determined using a β-galactosidase Enzyme Assay System (Promega) according to the manufacturer's protocol.

Statistical Analysis—Statistical analysis was performed using SPSS, version 11.0. Data with a normal distribution were expressed as means ± standard deviation (SD). Differences between the groups were determined using two-way analysis of variance (ANOVA) and the Student *t*-test. P values < 0.05 were considered to be statistically significant. Spearman's correlation test was used to determine the correlation between the numbers of ICP22 dense bodies and the SC-35 domain.

RESULTS

ICP22 Is Localized to Small Dense Nuclear Bodies and Is Paired with the SC-35 Domain—Observation of ICP22-transfected CHO cells under a fluorescence microscope revealed punctate expression of ICP22 in the nucleus (Fig. 2A), consistent with its previously reported localization to small dense nuclear bodies (*18*). Observation under a confocal microscope showed no overlapping between ICP22 and the SC-35 domain (Fig. 2C) that was detected by the SC-35 mAb (Fig. 2B). However, they seem to be paired in the nucleus (Fig. 2D). For further confirmation of this observation, more cells transfected with ICP22 were screened, and the numbers of punctate ICP22 and SC-35 were determined under a confocal microscope. Ten fields were selected at random, and 2–5 transfected cells were observed for counting in each field. The numbers of green (ICP22-GFP) and red (SC-35 stained) dots in the same transfected cell were determined and are listed in Table 1. The data shown in this table indicate that the numbers of ICP22 and SC35 stained were almost equal in a cell, even if the number of ICP22 and SC-35 pairs was different in different cells. Pearson's correlation coefficient $r = 0.98$ ($P < 0.001$).

ICP22 Localization in the Nucleus Requires Completion of the Main Structure of the 1–320th Amino Acids—In investigating the localization of different ICP22 mutants expressed in cells, although fusions of the green fluorescence protein, and mutants S1, S2, and S23 were observed in the cytoplasm and nucleus, however, they were mainly distributed in the nucleus. Mutant S3 was dispersed in the cytoplasm, and mutants M77 and S34 were dispersed in the nucleus. However, punctate expression of S12, S123, D30, and D24 was found in the nucleus, as was found for the complete ICP22 sequence (Fig. 3). These results suggested that ICP22's localization in the nucleus depended on the completion of its main structure except the C-terminal.

Viral Transcriptional Repression by ICP22 Requires Its Main Structure Except the C-Terminal—To compare the abilities of mutants with ICP22 to repress viral transcription *in vivo*, Western blotting was performed at first to check their expression in transfected CHO cells (Fig. 4A). Because it was difficult technically to obtain the complete ICP22 in the *E. coli* expression system, we only produced an antibody against the N-terminal peptide of ICP22 (1–36th amino acids), and used this antibody to analyze the expression of S1, S12, S123, and the complete ICP22 in CHO cells. As seen in the CAT assay, the complete ICP22 protein decreased CAT expression controlled by the HSV1 α-4 promoter by 90% (Fig. 4B), and mutants S123 and D30 were as equally effective as the complete

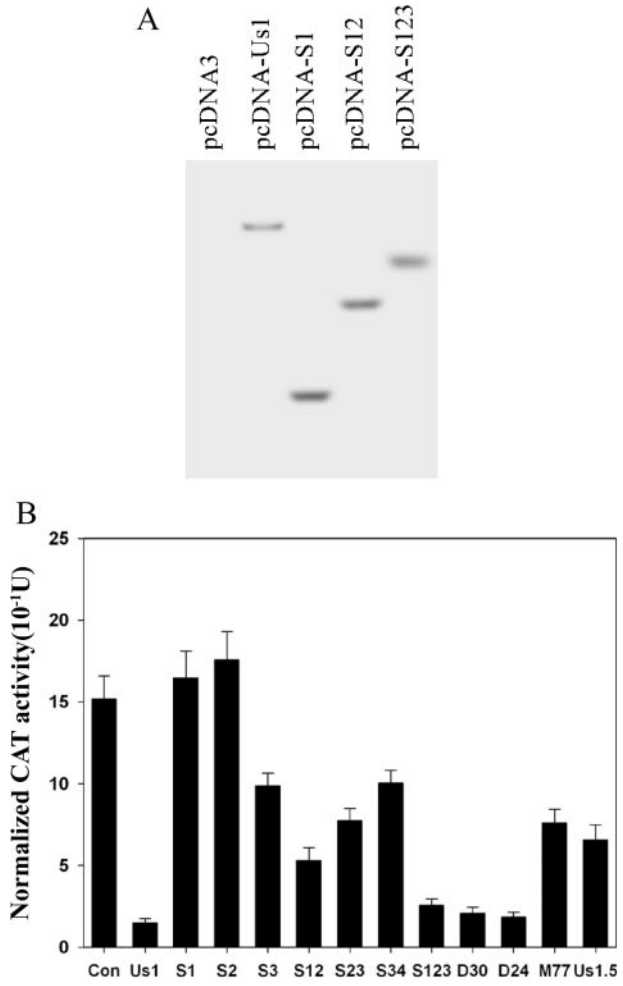

Fig. 4. **The ability of mutants of ICP22 to repress transcription in CHO cells.** (A) Photograph of an immunoblot showing the expression of different lengths of the ICP22-derived protein. CHO cells were transfected with pcDNA3, pcDNA-Us1, pcDNA-S1, pcDNA-S12 or pcDNA-S123, and the proteins were separated by electrophoresis, transferred to a nitrocellulose membrane, and then treated with mouse polyclonal antiserum against the ICP22 N-terminal fragment of 36aa. (B) Inhibition of CAT expression under the control of the upstream sequence of the HSV1 α4 gene by different ICP22 mutations. CHO cells were cotransfected with 1 μg of pCAT-332 (containing the upstream sequence of the HSV1 α4 gene) and 0.1 μg pCMVβ (Clontech), a galactosidase expression plasmid as an internal control for transfection efficiency, and 1 μg of pcDNA3 (Con), pcDNA-Us1 (Us1), pcDNA-S1 (S1), pcDNA-S2 (S2), pcDNA-S3 (S3), pcDNA-S12 (S12), pcDNA-S23 (S23), pcDNA-S34 (S34), pcDNA-S123 (S123), pcDNA-D30 (D30), pcDNA-D24 (D24) ,pcDNA-M77(M77), or pcDNA-us1, respectively. Cells were harvested 36h later and CAT activity was quantitated by liquid scintillation counting (LSC). One unit (U) was defined as the amount of enzyme required to transfer 1 nmol of acetate to chloramphenicol in one minute at 37°C. To normalize for transfection efficiency, the CAT expression level was normalized as to the level of β-galactosidase expression. The bars represent the average normalized CAT activity in extracts of transfected cells per well. The error bars indicate the SD of the mean (n = 6).

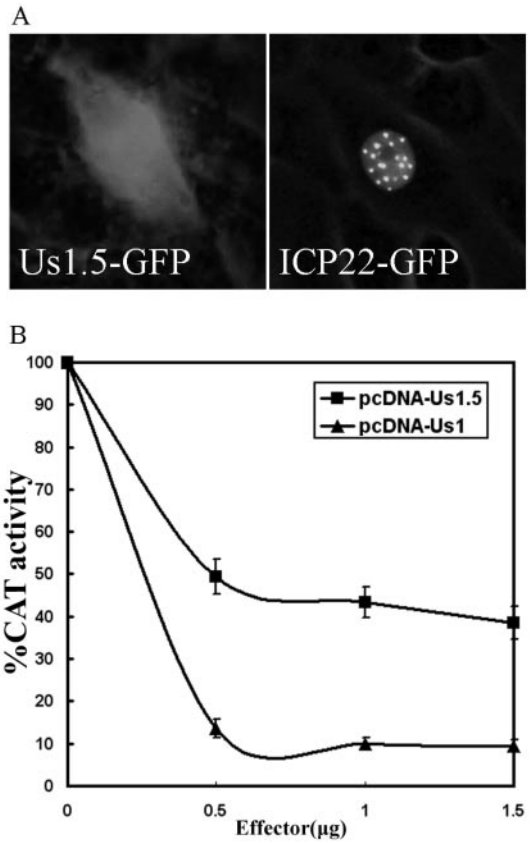

Fig. 5. **Localization and function of Us1.5 are distinct from those of ICP22.** (A) CHO cells were transiently transfected with pEGFPN-Us1.5 or pEGFPN-Us1 expressing the fusion proteins indicated and then analyzed 36 h posttransfection by fluorescence microscopy without fixation. Compared with the ICP22-GFP's punctuate in the nucleus, Us1.5-GFP was dispersed in the whole cell. (B) CHO cells were cotransfected with 1 μg of pCAT-332 (containing the upstream sequence of the HSV1 α4 gene) and an increased concentration of pcDNA-Us1.5 or pcDNA-Us1 as an effector. The squares represent the effect of pcDNA-Us1.5 or pcDNA3+pcDNA-Us1.5 on pCAT-332. The triangles represent the effect of pcDNA-Us1 or pcDNA3+pcDNA-Us1 on pCAT-332. The values shown are the percentages of acetylated chloramphenicol *versus* the concentration of the effector pcDNA-Us1.5 or pcDNA-Us1. To control for transfection efficiency, each of the reporter plasmids was cotransfected with the pCMVβ plasmid and the relative CAT activity was normalized for galactosidase activity. The error bars indicate the SD of the mean (n = 6). Compared with ICP22, Us1.5 showed a lower transcriptional repression effect.

protein (Fig. 4B), In contrast, M77, with a 2-amino-acid conservative substitution at the N terminal, decreased the expression by 50% (Fig. 4B). S3, S12, S23, and S34 causing similar repression (Fig. 4B) inhibited CAT activity by 35–65%. Interestingly, mutants S1 and S2 slightly enhanced the CAT expression.

Localization and Function of Us1.5 Are Distinct from Those of ICP22—Investigation of the localization of Us1.5 suggested that this protein is distributed in the cytoplasm and nucleus in a dispersed state like S34 (Fig. 5A). On CAT assaying of CHO cells co-transfected with pcDNA-Us1.5 and pCAT-332 *versus* ICP22, Us1.5 only showed a lower transcriptional repression effect (Fig. 5B). This result supports the observation described above.

DISCUSSION—Based on the fact that ICP22 is localized to small dense nuclear bodies and is semihomologous to Us1.5, we hypothesized that its structure is related to

its multifunctional role in viral replication. In this study, the entire encoding sequences of ICP22 and Us1.5, as well as several partial ICP22 sequences, were used to investigate this potential relationship between structure and biological function. Interestingly, the results obtained on confocal microscopy show that ICP22 is localized to small dense nuclear bodies being paired with the SC-35 domain detected by specific immune fluorescence antibodies. The screening of all stained cells supports this observation even though the cells in different cycle phases show different numbers of paired ICP22 and SC35. Generally speaking, the SC-35 domain is thought to be concentrated in nuclear bodies containing mRNA metabolic factors—sometimes referred to as "speckles" (*23–25*). Thus, the present finding increases our understanding of ICP22 localization, and strengthens our earlier findings suggesting that ICP22 is involved in repression of cellular transcription and regulation of gene transcription (Cun, W. and Li, Q., unpublished data).

The results of these experiments also demonstrate that the complete main structure of ICP22 except the C-terminal is necessary for its localization in a punctate state within small dense nuclear bodies. As shown in previous work, the nuclear localization signal of ICP22 is contained within its 1–131 amino acid sequence (*18*), suggesting that this peptide confers the ability to enter the nucleus. In addition, we found in the current study that mutants S1, S2, S3, S23, and S34 did not show punctate formation in the nucleus, and mutant M77, which had only two changed amino acids, was dispersed in the nucleus. These findings indicate that amino acids 1–220 (see the effect of mutant S12, for example) are necessary for punctate formation.

An obvious implication of these results is that completion of the main ICP22 structure (1–320th amino acids) may determine its biological role, which is related to its nuclear localization. Further analysis confirmed that this completion of the main structure of ICP22 determined its strong ability to repress transcription in the CAT assay system. These results suggest that main structural completion of the sequence (within 320 amino acids) is required for ICP22's characteristic localization and is probably related to its function in viral infection. However, the C-terminal (after the 320th amino acid) appears unimportant for this characteristic. These data help to deepen our understanding of the biochemical mechanism of ICP22 in viral replication.

Another interesting finding in this study is that the nucleotidylylation site in ICP22 determines, to some extent, its nuclear localization and transcriptional repression in the CAT assay. Mutation at this site (RRAP→RKLP) completely changes these two characteristics. Although the mechanism underlying nucleotidylylation in viral proteins has not yet been accurately explained (*14*, *15*), nucleotidylylation in immediate-early HSV1 proteins has been confirmed to be an important structural characteristic (*26*). This suggests that nucleotidylylation of ICP22 could be potentially significant as to its function in viral replication.

If completion of the main structure, which comprises the 1–320th amino acids, is required for ICP22's characteristic nuclear localization and transcriptional repression, then Us1.5 should exhibit a different cellular distribution and another biological function. Our further investigation confirmed this. Despite possessing the same amino acid sequence as the ICP22 carboxyl-terminal, Us1.5 was inferred to perform a specific role during viral replication. These results seemingly suggest that Us1.5 functions differently from ICP22 during viral infection, *via* a currently unknown mechanism that needs further investigation.

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