The Products of Gene Us11 of Herpes Simplex Virus Type 1 Are DNA-binding and Localize to the Nucleoli of Infected Cells

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SUMMARY

We have used antisera raised against synthetic oligopeptides to characterize the protein products from herpes simplex virus type 1 gene Us11. These antisera recognized predominantly polypeptides of apparent molecular weight 21000 and 22000, but also polypeptides of apparent molecular weight 17500, 15000, 14000 and 11000. Tryptic peptide fingerprint analysis confirmed that these polypeptides were all closely related. The 21000 and 22000 molecular weight polypeptides were shown to be DNA-binding proteins, and immune electron microscopy demonstrated their strong localization within nucleoli of infected cells.

INTRODUCTION

Herpes simplex virus (HSV) has a linear double-stranded DNA genome with an approximate molecular weight of 100 × 10⁶ (Becker et al., 1968). The genome consists of a long (L) and a short (S) region, each comprising a unique sequence (UL, Us) flanked by inverted repeats (TR₁/IR₁, TRₛ/IRₛ; Sheldrick & Berthelot, 1974). A direct repeat sequence known as the 'a' sequence is present at the genomic termini and in inverted orientation at the L-S junction. One to several copies of the 'a' sequence may be present at the L-S junction and at the L terminus, but only one copy is usually found at the S terminus (Wagner & Summers, 1978; Locker & Frenkel, 1979).

The 'a' sequence is thought to be involved in a number of aspects of the virus life cycle. Inversion of the L and S segments can occur between the inverted copies of the 'a' sequence to generate the four isomeric forms of viral DNA which are found in equimolar amounts (for review, see Roizman, 1979). The 'a' sequence contains signals for the site-specific cleavage events which generate unit length monomers from the long linear concatemers produced during viral DNA replication (Jacob & Roizman, 1977; Jacob et al., 1979; Mocarski & Roizman, 1982; Varmuza & Smiley, 1985), and it contains the signals required for the packaging of correctly sized DNA molecules into viral particles (Stow et al., 1983). In addition, the 'a' sequence contains the promotor-regulatory domain for a gene within the adjacent long repeat region (Chou & Roizman, 1986).

Upon infection of cells with HSV at least 17 DNA-binding proteins are induced (Bayliss et al., 1975; Purifoy & Powell, 1976). Two such proteins, of apparent mol. wt. 21000 and 22000 (21K and 22K), appear to interact specifically with the 'a' sequence of HSV-1 (Dalziel & Marsden, 1984). Therefore we were interested in characterizing these proteins further, since they could potentially be involved in 'a' sequence functions.

Proteins of apparent mol. wt. 21K and 22K are produced in abundance late in infection and are encoded by the short unique region of the genome (Marsden et al., 1976, 1978). Hybrid-arrested translation experiments (Rixon & McGeoch, 1984) suggested that gene Us11 which lies in the short unique region (McGeoch et al., 1985) might encode at least the 21K polypeptide, and this was confirmed using an antiserum raised against a synthetic oligopeptide representing the carboxy-terminal seven amino acids of the predicted polypeptide product of gene Us11 (Johnson et al., 1986). The latter study also showed that Us11 belongs to the true late class of genes. The
predicted product of gene U₅₁₁ has a highly unusual amino acid composition. The carboxy-
terminal portion of the protein contains 24 tandem repeats of the sequence X-Pro-Arg, where X
is any one of nine amino acids, Ala, Asp, Gln, Glu, Ile, Pro, Ser, Thr or Val (Rixon & McGeoch,
1984). The repeating unit represents approximately 45% of the protein (see Fig. 1). The high
arginine content renders this a very basic protein, consistent with it being a DNA-binding
protein.

It is highly likely that the above properties all relate to the same 21K and 22K polypeptides,
and that the ‘a’ sequence-specific DNA-binding proteins are the product of gene U₅₁₁. In this
communication we show that the 21K and 22K products of gene U₅₁₁ are DNA-binding
proteins, and that they localize very strongly to nucleoli within infected cells.

METHODS

**Virus.** HSV-1 strain 17syn⁺ (Brown et al., 1973) and HSV-2 strain HG52 (Timbury, 1971) were grown in
BHK-21 C13 cells (Macpherson & Stoker, 1962).

**Radioisotopic labelling of infected cells.** Cell monolayers at approximately 80% confluence were infected for 1 h
with 20 p.f.u. per cell. Unadsorbed virus was removed and the cells maintained in Eagle’s medium containing one-
fifth the normal concentration of methionine and 2% calf serum. Three h later [³⁵S]methionine (Amersham; sp.
act. > 1000 Ci/mmol) was added at 50 μCi/ml.

**Preparation of infected cell extracts.** Cultures were harvested at 24 h post-infection. For immunoblotting infected
cells were washed twice with phosphate-buffered saline (PBS) and suspended in denaturing buffer (0.05 M-Tris-
HCl pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol and enough bromophenol blue to visualize the dye
front) at a concentration of 1 x 10⁶ cells/ml. The extracts were stored at -70 °C until use.

For immunoprecipitation infected cells were washed twice with PBS, once with water and then resuspended in
water at 1 x 10⁶ cells/ml. An equal volume of lysis buffer (40 mM-Tris–HCl pH 8.2, 4 mM-NaCl, 2 mM-EDTA,
2 mM-2-mercaptoethanol) was then added and the mixture incubated at 0 °C for 30 min and then centrifuged at
100000 g for 1 h. The extract was dialysed extensively against B2 buffer (20 mM-Tris–HCl pH 8.0, 50 mM-NaCl,
1 mM-EDTA, 1 mM-2-mercaptoethanol, 10% glycerol) at 4 °C, then centrifuged at 10000g for 30 min. The
clarified supernatant was stored at -70 °C.

**Generation of anti-peptide sera.** The peptides NH₂-Tyr-Met-Ser-Gln-Thr-Gln-Pro-Pro-Ala-Pro-Val-Gly-COOH,
NH₂-Tyr-Arg-Gly-Asp-Asp-Asp-Pro-Pro-Ala-Gly-Gln-Cys-COOH, and NH₂-Tyr-Pro-Arg-Glu-Pro-Arg-Thr-
Ala-Gly-Ser-Val-COOH were synthesized by Cambridge Research Biochemicals, Cambridge, U.K. These
correspond to the amino-terminal 11 amino acids (N₁₁), amino acids 48 to 58, and the carboxy-terminal 11 amino
acids (C₁₁), respectively, of the predicted product of gene U₅₁₁, with an N-terminal tyrosine residue added to
facilitate coupling to bovine serum albumin (BSA).

New Zealand White rabbits were immunized intramuscularly with the BSA-conjugated peptides emulsified in
Freund’s complete adjuvant for the first injection and then in Freund’s incomplete adjuvant for subsequent
injections. Rabbits were bled 10 days after each injection. The specificity of the antisera for the relevant peptide
and the effective removal of anti-BSA antibodies following absorption with BSA immobilized on Sepharose 4B,
were established by radioimmunoassay.

The sera used in this study are designated by a number which is unique to the rabbit from which a particular
antisera was obtained. Rabbits 14327 and 14328 were immunized with the C₁₁ peptide, rabbit 14473 with the
N₁₁ peptide and rabbits 14060 and 18416 with the ‘48 to 58’ peptide. The previously described serum 12848 was
raised against the carboxy-terminal seven amino acids of the predicted U₅₁₁ gene product (Johnson et al., 1986).

**Immunoblotting.** Immunoblotting was carried out as described by Towbin et al. (1979) with slight modifications
(Haarr et al., 1985). Briefly, labelled proteins were separated electrophoretically on 5 to 12.5% SDS–
polyacrylamide gels (Marsden et al., 1978) and transferred to nitrocellulose strips. The strips were incubated
overnight at 65 °C in the presence of 0.05% Tween 20, then reacted with antiserum for 2 h at 37 °C. After washing,
bond antibody was detected using ¹²⁵I-labelled Protein A.

**Immunoprecipitation.** One hundred μl of infected cell extract was mixed with an equal volume of buffer
containing 0.2 M-Tris–HCl pH 8.0, 1% NP40, 1% sodium deoxycholate, 10% glycerol and 0.4 mM-phenylmethyl-
sulphonyl fluoride (Sigma), and then incubated with 25 μl of antiserum, in the presence or absence of peptide, for
1 h at 37 °C. Immune complexes were precipitated by the addition of Protein A-Sepharose (Sigma), washed
several times and the bound proteins were then eluted with denaturation buffer (see above) and analysed on 5 to
12.5% SDS–polyacrylamide gels.

**Immune electron microscopy.** Samples were prepared as described previously (Frame et al., 1986). Grids,
containing sections through infected or mock-infected cells, were submerged in antiserum diluted 1 in 50 in PBS
containing 0.05% Tween 20 (PBS/T20) in the absence or presence of various amounts of peptide for 45 min at
room temperature, jet-washed with PBS/T20, then further incubated in a 1 in 50 dilution of goat anti-rabbit IgG
HSV-1 Usll gene products

1923

coupled to gold (10 nm diam. particles) (Miles Laboratories) in PBS/T20 for 45 min. The grids were jet-washed as before, with a final wash in deionized water and then dried. Contrast was enhanced by exposing the sections to OsO₄ vapour for 2 h and the sections were examined using a Jeol 100S electron microscope.

Preferential staining of ribonucleoproteins was carried out according to Bernhard (1969). Briefly, sections which had not been exposed to OsO₄ were stained for 2 min in 0.05% aqueous uranyl acetate, destained for 10 min with various concentrations of EDTA and then post-stained for 1 min with lead citrate. The grids were jet-washed extensively between each step, using deionized water.

Tryptic peptide mapping. HSV-1-infected extracts were subjected to phosphocellulose chromatography and the fractions containing the 21K polypeptide were pooled and concentrated. The proteins were electrophoretically separated on 10% SDS-polyacrylamide gels. A slice from one end of the gel was removed for immunoblotting and the rest dried immediately after electrophoresis. Four radioactive spots were placed at the corners of the gel to facilitate alignment with their autoradiographs. Such alignment allowed identification of the relevant bands, and these were then cut out and the polypeptides eluted by the method of Anderson et al. (1973). They were desalted by passage through Sephadex G-25 and the SDS was removed by the method of Henderson et al. (1979). The pellet from this procedure was resuspended in 100 μl of a 1% solution of ammonium bicarbonate containing 10 μg of TPCK-treated trypsin. After 16 h at 37 °C a further 1 μg of TPCK-treated trypsin was added and incubation continued for 4 h. The peptides were lyophilized, oxidized with performic acid (Hirs, 1967), then diluted 50-fold with water and lyophilized. Peptides in pH 2.1 electrophoresis buffer (acetic acid : formic acid : water, 8 : 2 : 90) were applied to a spot 4 cm from each of two adjacent edges of 20 x 20 cm Eastman cellulose chromatogram sheets (no. 13255). After electrophoresis for 45 min at 600 V the chromatogram was dried in a current of cold air and peptides were separated in the second dimension by ascending chromatography in water: butanol: pyridine: acetic acid, 24:30:20:6. Dried chromatograms were sprayed with En3Hance (New England Nuclear) and exposed at -70 °C to Kodak X1 film.

RESULTS

Antisera to synthetic oligopeptides recognize several different mol. wt. polypeptides

Antiser were raised against synthetic oligopeptides representing the amino-terminal 11 amino acids, the carboxy-terminal 11 amino acids and an internal region from amino acids 48 to 58, inclusive, of the Usll gene product (Fig. 1). All three peptides induced antisera that reacted with proteins of the appropriate mol. wt. in both immunoprecipitation and immunoblotting experiments. Fig. 2 shows an example of an immunoprecipitation experiment. The antiserum against the carboxy-terminal peptide (anti-C₁₁ serum) specifically recognizes a 21K/22K doublet (lane 3) which is not precipitated by preimmune serum (lane 2). Although the background in these immunoprecipitation experiments was high, probably due to the weak nature of the interaction between the antipeptide antibodies and the protein, specificity was demonstrated by the addition of the carboxy-terminal peptide which blocks the precipitation of the 21K/22K polypeptides without significantly affecting the precipitation of other proteins (lane 4). High concentrations of peptide caused an overall reduction in the intensity of bands (lane 5). This control also showed that a 14K polypeptide was also specifically precipitated. This is interesting, as no strong 14K band was evident in the HSV-1 extract and suggests either that the antiserum had a very high affinity for this polypeptide, or that this polypeptide was generated during the immunoprecipitation reaction. No experiments were done to distinguish between these possibilities. The antiserum against the amino-terminal peptide (anti-N₁₁ serum) also precipitated a 21K/22K doublet but did not detectably precipitate the 14K polypeptide (lane 6).

Immunoblotting experiments showed that the anti-C₁₁ serum recognized not only the 21K, 22K and 14K polypeptides but also polypeptides with apparent mol. wt. 17.5K and 11K (Fig. 3 and 4a). The 11K polypeptide often appeared as a doublet. A faint band can be seen in the region of 12K in Fig. 3, but there was often a weak reaction in this region in mock-infected extracts also (see Fig. 4) suggesting that it is non-specific. However, a number of observations suggest that the 17.5K, 14K and 11K polypeptides are related to 21K/22K. First, they increased in intensity in parallel to 21K/22K through time-course experiments (Johnson et al., 1986); second, they were recognized only in HSV-1-infected cell extracts and not in mock-infected or HSV-2-infected cell extracts (Fig. 4a, lanes 1 to 3); and third, they were not recognized by preimmune sera, which generally produced relatively clean blots (for example Fig. 7, lane 5).
Fig. 1. Schematic diagram of the Usl1 gene product. The predicted product is a 161 amino acid protein with an estimated mol. wt. of 17.8K (Rixon & McGeoch, 1984) and has the unusual feature of containing 24 tandem repetitions of the sequence X-Pro-Arg towards the carboxy terminus. The amino acid sequences of the three synthetic oligopeptides used to raise antisera and corresponding to the amino-terminal 11 amino acids (Nt 1), the carboxy-terminal 11 amino acids (Ct t), and amino acids 48 to 58, respectively, are shown. An additional amino-terminal tyrosine residue was added to each sequence to facilitate coupling to BSA.

The next section describes experiments which establish more clearly the nature of the relationship. Non-specific binding often occurred in the region of the 14K band with several immune and preimmune sera, but it was usually much weaker than the reaction seen with the carboxy-terminal antisera, and also appeared in mock-infected extracts (Fig. 4a, compare lane 1 with lanes 4 to 6). Sometimes this reaction was very weak (e.g. Fig. 4a, lanes 5 and 6), but in other experiments the reaction was stronger (e.g. Fig. 4c, lanes 1 and 2).

The anti-N11 serum also recognized 21K and 22K (Fig. 4a, lane 4; Fig. 4b, lane 1) and immunoblotting in the presence of specific peptide, but not non-specific peptide, inhibited this interaction (Fig. 4b). Two lower mol. wt. polypeptides of approximately 12K and 14K (marked with an open circle in Fig. 4b) appeared to represent a non-specific interaction, as they were not inhibited by the relevant peptide (Fig. 4b, lane 2).

Antisera against the internal oligopeptide (anti-'48 to 58' serum) specifically recognized the 21K and 22K polypeptides (Fig. 4c). In addition, one antiserum recognized a 17.5K polypeptide not detected in control extracts, while another recognized polypeptides in the region of 14K and 15K also not detected in control extracts (lanes 1 to 3 and 5 to 7, respectively).

The lower mol. wt. polypeptides and the 21K/22K products of gene Usl11 have common amino acid sequences

To investigate further the relationship between the lower mol. wt. polypeptides and the 21K/22K products of gene Usl11 they were individually subjected to tryptic peptide analysis. The results (Fig. 5) clearly confirm that the 22K, 21K, 17.5K, 15K, 14K and 11K polypeptides have common amino acid sequences. The 21K and 22K polypeptides differed mainly in the presence of an additional strong spot in 22K (spot 4), and two other additional minor spots, seen at the top left of the gel. These were present to varying extents in the lower mol. wt. polypeptides, but their intensity relative to the other spots was reduced. The lower mol. wt. polypeptides were strikingly similar to 21K, all the major spots being present, although their relative intensities might have altered. The 14K sample also contained a number of spots apparently unrelated to 21K (e.g. spots a to d), but the region of the gel from which this polypeptide was excised contains mock-infected cell polypeptides and it is likely that the additional spots derived from these.

The 21K Usl11 gene product is a DNA-binding protein

We have previously shown that the 21K and 22K proteins encoded by gene Usl11 are regulated as true late gene products (Johnson et al., 1986). To determine whether the late 21K/22K Usl11 gene products are related to the late 21K/22K DNA-binding proteins, we purified the latter by DNA-cellulose chromatography and tested them for reaction with the anti-peptide sera.
HSV-1 U₃₁₁ gene products

Fig. 2. Immunoprecipitation with gene U₃₁₁ anti-oligopeptide serum. Proteins labelled with [³⁵S]methionine were precipitated from an HSV-1-infected cell extract (lane 1) by either pre-immune serum from rabbit 14327 (lane 2), anti-C₁₁ serum 14327 incubated in the presence of 0, 1 or 10 μg of C₁₁ peptide (lanes 3 to 5, respectively) or anti-N₁₁ serum 14473 (lane 6). Bands which appear to be specifically precipitated are marked (■).

Fig. 3. Immunoblotting with the anti-C₁₁ (U₃₁₁) serum. Lane 1 shows ³⁵S-labelled HSV-1-infected cell proteins transferred to nitrocellulose. Lane 2 shows the polypeptides on the nitrocellulose that are detected by the anti-C₁₁ serum 14328. Alignment of the two lanes allows the apparent mol. wt. of the polypeptides to be identified (Haarr et al., 1985).
Fig. 4. Comparison of the polypeptides recognized in immunoblotting experiments by the different anti-peptide sera. (a) Anti-C₁₁ serum 14327 (lanes 1 to 3) and anti-N₁₁ serum 14473 (lanes 4 to 6) were reacted in immunoblotting experiments against extracts from HSV-1-infected cells (lanes 1 and 4), HSV-2-infected cells (lanes 2 and 5) and mock-infected cells (lanes 3 and 6). The polypeptides were separated on 5 to 12.5% polyacrylamide gels, transferred to nitrocellulose and reacted with the antisera. (b) Anti-N₁₁ serum 14473 was reacted against HSV-1-infected cell extracts either alone (lane 1) or in the presence of 100 μg of N₁₁ peptide (lane 2) or of C₁₁ peptide (lane 3). (c) Anti-'48 to 58' serum 14060 (lane 1) and serum 18416 (lane 2) were reacted against extracts from mock-infected cells (lanes 1 and 5), HSV-2-infected cells (lanes 2 and 6) and HSV-1-infected cells (lanes 3 and 7). Anti-C₇ serum 12848 (raised against an oligopeptide corresponding to the carboxy-terminal seven amino acids of gene U₉₁₁) reacted against extracts from HSV-1-infected cells is shown for comparison (lane 4). Bands detected by the anti-N₁₁ serum in all cell extracts, and not inhibited by peptide, are indicated by an open circle (O) in (b).
Fig. 5. Tryptic peptide analysis of polypeptides recognized by anti-peptide sera. Tryptic peptide fingerprints were obtained for each of the previously identified polypeptides. The tryptic peptide digestion products from 21K were mixed with each of the other samples before chromatography, to verify comigration of the apparently identical spots.
Immunoblotting experiments (Fig. 6) confirmed that the 21K and 22K products of gene U₃₁₁ are indeed DNA-binding proteins. A band in the region of 17.5K was detected on one occasion (lane 3) but faint bands in this region were also present in the mock-infected control (lane 1) and so the specificity of this reaction is uncertain. The inability to detect the lower mol. wt. polypeptides does not necessarily imply that they did not bind to DNA, since they were not detected in the high salt extract originally applied to the DNA-cellulose columns (lane 4).

**Localization of the U₃₁₁ gene product**

We analysed the subcellular localization of the U₃₁₁ gene products by immune electron microscopy using the anti-oligopeptide antisera. Fig. 7 shows some examples of the pattern of
labelling seen with the anti-C11 antiserum. The most striking observation is the intense labelling of heavily stained (osmophilic) structures within the nucleus (Fig. 7a, c). These were readily distinguishable from the marginated chromatin (Fig. 7c, d) and were invariably sites of strong localization. These structures are a well recognized feature of HSV-infected cells and constitute one of the residual components of the nucleolus, which disaggregates following HSV infection (Sirtori & Bosio-Bestetti, 1967; Schwartz & Roizman, 1969; Dupuy-Coin et al., 1978). Their designation as nucleoli was confirmed using differential staining techniques that preferentially stain ribonucleoproteins (Bernhard, 1969; Fig. 7b, d). Fig. 7(b) is an example of such preferential staining, in the absence of immunogold labelling, and shows a nucleolus embedded within chromatin (a common location, see also for example Fig. 7c, d) at 2 h post-infection, i.e. before the nucleoli have completely disaggregated (Schwartz & Roizman, 1969). In Fig. 7(c) the nucleolus is a floccular structure, slightly less osmophilic than the marginated chromatin in which it is embedded. In contrast, following the preferential staining of ribonucleoproteins, this floccular structure can clearly be distinguished from the marginated chromatin (Fig. 7d). There was no obvious localization of gold particles to the marginated chromatin, or to dense bodies or viral capsids within the nucleus and the nucleoplasm had a uniformly low level of labelling (Fig. 7a, c, g). In contrast, labelling of the cytoplasm varied among individual cells, from a very low level, through intermediate stages, to the very intense labelling seen in Fig. 7(e). The gold marker was uniformly distributed over the cytoplasm of the labelled cells apart from the mitochondria which were unlabelled.

Identical patterns of localization to those described for the anti-carboxy-terminal serum were observed with antisera against the amino-terminal peptide. In both cases labelling was abolished by mixing the antibody with the homologous peptide but was not affected by the heterologous peptide (compare nl in Fig. 7f, g). Furthermore, similar localization patterns were not seen with preimmune sera or with antisera against other HSV proteins (results not shown). The results indicate that the localization pattern observed accurately reflects the distribution of the 21K/22K polypeptide and is not the result of non-specific interactions or chance cross-reaction of the antibodies with an unrelated cellular protein.

**DISCUSSION**

We have used anti-oligopeptide sera to define the products of gene Us11. These antisera specifically recognize proteins of apparent mol. wt. 21K and 22K which we have previously shown to be regulated as true late gene products (Johnson et al., 1986). In addition these antisera also recognize at least four lower mol. wt. polypeptides which were shown by tryptic peptide fingerprinting to be related to 21K and 22K (Fig. 5). However, the precise relationship between these products is unclear. Although the amino acid sequence of the predicted Us11 gene product contains 24 tandem repeats of the tripeptide X-Pro-Arg, there is no extensive reiteration within the DNA sequence, as all the potential codons for proline and arginine are used (Rixon & McGeoch, 1984). However, there is a short 18 bp sequence which is present as three tandem repeats in HSV-1 strain 17syn+ (Rixon & McGeoch, 1984), but only as two tandem repeats in HSV-1 strain Patton (Watson & Vande Woude, 1982). It has been suggested (Rixon & McGeoch, 1984) that variation in the number of repeats of this 18 bp sequence may account for the interstrain variability in the apparent mol. wt. of the 21K polypeptide (Vmw21) reported by Lonsdale et al. (1979). It is also possible that this could account for the difference between the 21K and 22K polypeptides.

Carboxy-terminal antisera recognize products of apparent mol. wt. 17-5K, 14K and 11K which amino-terminal antisera do not recognize. Antisera against the internal oligopeptide (amino acids 48 to 58) recognize the 17-5K and possibly the 14K polypeptides, but not the 11K polypeptide. At first sight this might suggest that the 17-5K, 14K and 11K products, while retaining the carboxy terminus, lack progressively larger regions from the amino terminus of 21K. However, this interpretation is not readily compatible with the distribution of the three methionine residues in the predicted Us11 gene product: these are all situated in the amino-terminal region (residues 1, 27 and 41 respectively).
One reason for studying the \( U_{11} \) gene products was to determine their relationship with the previously described 21K and 22K DNA-binding proteins shown to interact specifically with the 'a' sequence of HSV-1 (Dalziel & Marsden, 1984). We could not obtain the 'a' sequence-specific DNA-interacting proteins in sufficient quantity for immunoblotting or immunoprecipitation experiments, and so direct experimental evidence was not achieved. However, by immunoblotting proteins eluted from DNA-cellulose columns we could confirm that the 21K and 22K \( U_{11} \) gene products were DNA-binding proteins (Fig. 6). The existence of a second, completely unrelated, pair of late 21K/22K DNA-binding proteins would seem so unlikely as to suggest strongly that the 21K/22K proteins which specifically interact with the HSV-1 'a' sequence are also the products of gene \( U_{11} \).

From the electron micrographs shown in Fig. 7 it is clear that there are two areas of the cell in which the \( U_{11} \) gene products localize, namely the cytoplasm and the nucleolus. The cytoplasmic labelling varied in intensity among individual cells but the reason for this is not apparent. Nor is it clear what this behaviour reveals about the function of the abundant 21K/22K DNA-binding proteins.

The structure and function of nucleoli has recently been reviewed (Sommerville, 1986). The sole function of the nucleolus appears to be the generation and maturation of ribosomal particles. Nucleoli are therefore generated around ribosomal chromatin and are often surrounded and penetrated by so-called nucleolus-associated chromatin (Jordan, 1984). This is seen quite clearly in Fig. 7, for example in (b) and (c).

Studies on the effects of HSV infection on cellular metabolism have led to the conclusion that the nucleolus disaggregates to produce spherical densely staining components (dense bodies) and a less densely staining, irregularly shaped, granular component (Sirtori & Bosio-Bestetti, 1967; Schwartz & Roizman, 1969; Dupuy-Coin et al., 1978). In contrast to the granular component, the dense bodies contain no DNA or RNA, although they have been shown to contain at least one nucleolus-specific protein (Puvion-Dutillleul et al., 1985). No association was found between the \( U_{11} \) gene products and dense bodies.

The predominant association of the \( U_{11} \) gene products with the granular component of the nucleolus, the site of production, processing and maturation of pre-ribosomal particles
HSV-1 U₅₁1 gene products
Fig. 7. Immunolocalization of the U6.11 gene products. Electron micrographs of thin sections through HSV-1-infected BHK C13 cells, harvested at 12 h post-infection (a, c to g) or 2 h post-infection (b). The sections were incubated with anti-C11 serum 14327 either alone (a, c to e) or in the presence of 1 μg of C11 peptide (f) or N41 peptide (g). Preferential staining of ribonucleoproteins was carried out on sections (b) and (d), destaining with 40 mM-EDTA (b) or 80 mM-EDTA (d). Bar markers represent 1 μm (a, b, e to g) or 0.5 μm (c, d). NUC, nucleus; CYT, cytoplasm; nl, nucleolus; d, dense body; m, marginated chromatin. In (e), CYT1, CYT2, CYT3 represent the cytoplasm of three adjacent cells.
(Sommerville, 1986), appears rather similar to that previously shown for ribocharin, a protein believed to be involved in nucleocytoplasmic transport of ribosomes (Hugle et al., 1985a) and S1, a structural component of ribosomal particles (Hugle et al., 1985b). This might suggest some preferential association of the US11 gene products with ribosomal RNA or ribonucleoproteins in infected cell nuclei, although an association with ribosomal DNA cannot be excluded. Disaggregation of the nucleolus and inhibition of ribosomal RNA synthesis occur early following infection (Schwartz & Roizman, 1969; Wagner & Roizman, 1969), and therefore it would seem unlikely that the US11 gene products play a major role in these events.

Nii et al. (1968) have previously shown localization of virus-induced antigens to similar structures using ferritin-conjugated antibodies. However, since only general anti-HSV sera were used, the identity of the antigens recognized remains unknown. Therefore, whether other viral antigens accumulate within these residual nucleoli is unclear.

Although the 21K/22K polypeptides appear to interact with the HSV-1 ‘a’ sequence in vitro, their nucleolar localization suggests that they may not bind to viral DNA in vivo, since viral DNA is located in the nucleoplasm but is specifically excluded from nucleoli (Rixon et al., 1983; Puvion-Dutilleul et al., 1985; Randall & Dinwoodie, 1986). Similar nucleolar localization has not been observed for other HSV-specified DNA-binding proteins, for example IE175, ICP8 (the major DNA-binding protein) and the DNA polymerase (Randall & Dinwoodie, 1986). In these cases the distribution reflects that of the virus DNA, i.e. localization to the nucleoplasm and exclusion from the nucleolus. The different patterns of localization of the virus DNA and the 21K protein suggests that 21K plays no direct role in virus DNA metabolism. This suggestion is supported by the recent isolation of a number of HSV mutants which lack gene US11 (Longnecker & Roizman, 1986; Umene, 1986; Brown & Harland, 1987) thereby demonstrating that this gene is not essential for virus replication in the tissue culture systems studied so far. Whether the US11 gene products influence the host-range dependence of the virus, or whether they play a role in latency or pathogenesis in vivo, remains to be determined. In vivo studies with the deletion mutants are awaited with interest.

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