Chromosome Damage Induced by Herpes Simplex Virus Type 1 in Early Infection

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SUMMARY

The chromosome damage induced by herpes simplex virus type 1 (HSV-1) in vitro was examined up to 6 h after infection using HT-1080 cells. Initial damage occurring within 3 h was specific, involving uncoiling of chromosome 1q12-21 and to a lesser extent the pericentric regions of chromosomes 9, 16 and satellited chromosomes. For the initial unwinding, synthesis of the immediate early class of HSV proteins needed to occur as was demonstrated using HSV-1 temperature-sensitive mutants tsK and tsB7 and two viral inhibitors, β-propiolactone and psoralen plus long wavelength u.v. light. Later damage included chromatid breaks, acentric fragments and pulverization which did not take place until synthesis of delayed early proteins had begun.

It has long been known that one of the consequences of infection of mammalian cells by viruses is chromosome damage. Many RNA and DNA viruses induce a wide range of aberrations in vivo and in vitro (for review, see Harnden, 1974). Some viruses cause random aberrations whilst others induce specific alterations to one or more of the chromosomes. For example, adenovirus type 12, an oncogenic serotype, causes uncoiling in chromosome 17 and in specific sites of chromosome 1 (Steffensen et al., 1976), whereas adenovirus types 2 and 7, non-oncogenic serotypes, induce random aberrations. Herpes simplex virus (HSV) was initially reported to induce random damage in Chinese hamster cells (Hampar & Ellision, 1961). HSV infection was characterized by chromatid breaks, fragmentation, enhanced secondary constructions and pulverization, the degree of damage being related to the length of exposure of the cells to the virus. The mechanism by which HSV induces chromosome damage in infected cells is not understood although alteration in cellular DNA synthesis, lysosomal damage (Allison & Paton, 1965) or the effect of a viral capsid or virus-encoded protein (O'Neil & Rapp, 1971) have been suggested as positive causes.

Since aberrations are induced in HSV-infected cells before the onset of viral DNA synthesis (Waubke et al., 1968; Donner & Gönczöl, 1971) but not in cells in which protein synthesis is inhibited by puromycin (Donner & Gönczöl, 1971), synthesis of early viral proteins would appear to be necessary for the induction of chromosome damage. HSV gene expression is under temporal regulation with a cascade synthesis of three classes of polypeptides, α, β and γ. The synthesis of the α or immediate early (IE) class of polypeptides peaks 3 to 4 h after infection and declines thereafter (Honess & Roizman, 1974) as the rate of β polypeptide synthesis increases. The available evidence from studies using metabolic inhibitors implicates the IE proteins in the induction of chromosome damage by HSV. In this study, we have re-examined the role of HSV in the induction of chromosome damage by infecting cells with virus inactivated by different methods, and by using virus temperature-sensitive (ts) mutants which are blocked at different stages of infection.

Confluent monolayer cultures of HT-1080 cells (Rasheed et al., 1974) were used because of
Fig. 1. HT-1080 cells 6 h after infection with (a) no virus (M, marker chromosome), (b) wild-type HFEM at 5 p.f.u./cell (closed arrowheads indicate chromatid breaks; open arrowheads indicate unwinding of pericentric regions) or (c) tsK at 5 p.f.u./cell (arrowheads indicate unwinding of chromosome 1). (d) Higher magnification of uncoiled regions of chromosome 1 from (c).

their high growth rate and high mitotic index. These cells have a modal number of 46 with a consistent marker chromosome t(11:14q) (Fig. 1a), and other chromosome rearrangements such as a 14 isochromosome and monosomy for 17. MRC-5 and Flow 5000 fibroblast cell lines were also used to confirm that the observations were not limited to a particular cell line.

Wild-type HFEM (STH3) was obtained from A. C. Minson (University of Cambridge, Cambridge, U.K.) tsK (strain 17) was a gift from J. C. M. Macnab (University of Glasgow, Glasgow, U.K.), and tsB7 a gift from A. Buchan (University of Birmingham, Birmingham, U.K.). Virus stocks were grown on BHK-21 cells at 37 °C for wild-type HFEM virus and at 31.5 °C for ts mutant viruses. Virus titres were obtained using both baby hamster kidney (BHK) cells and HT-1080 cells at permissive (31 °C) and non-permissive temperatures (38.5 °C). A 105-fold reduction in the titre was obtained with ts viruses grown in HT-1080 cells at 38.5 °C. Strict temperature control was maintained by submerging cultures in a water-bath.

To analyse chromosomes, cells were halted at metaphase by exposure to colcemid (Gibco) at 0.1 μg/ml for 2 h. After harvesting the cells in 0.25% trypsin in 0.01% EDTA, they were prepared
Short communication

Table 1. *Proportion (%) of cells exhibiting chromosome damage 6 h post-infection with wild-type HFEM, tsK or tsB7*.

<table>
<thead>
<tr>
<th>Chromosome aberration</th>
<th>tsK†</th>
<th>tsB7</th>
<th>HFEM</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6</td>
<td>98</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Unwinding only</td>
<td>94</td>
<td>0</td>
<td>29</td>
<td>0</td>
</tr>
</tbody>
</table>
| Unwinding plus chromatid breaks, acen
tric fragment and pulverization | 0    | 2    | 66   | 0       |
| Mitotic index (%)     | 5    | 1    | 1    | 5       |

* Five × 10⁵ cells were plated out 48 h prior to infection and grown to subconfluence. All experiments were carried out at 38.5 °C in order to make direct comparisons. Five p.f.u./cell of virus was added to the cells and adsorbed for 1 h, the inoculum was removed and prewarmed medium was added for a further 6 h. Chromosomes were analysed as described in the text.

† 87% unwinding in chromosome 1, 4% in chromosomes 1 and 9, 3% in chromosomes 1, 9 and 16.

for examination and G-banded using a modification of Seabright (1971). The level of chromosome damage was calculated by analysing at least 50 metaphases from each treatment.

Using HFEM (STH₂) (as described in Table 1) the first damage seen to the chromosomes 3 h after infection was uncoiling of chromatin (despiralization) in the pericentric regions of chromosomes 1, 9 and 16 and some despiralization of the satellites on chromosomes 13, 14, 15, 21 and 22. At 6 h post-infection a range of aberrations (Table 1) was exhibited including uncoiling, extensive non-specific chromatid breaks (Fig. 1b), acentric fragments and a small, but consistent fraction of pulverized metaphases. No difference was observed between HSV-induced damage at 37 °C and 38.5 °C. Although HSV-induced aberrations have been reported to differ in both type and number in different cell lines (O'Neill & Miles, 1969), we obtained identical results using the malignant, immortal HT-1080 cell line and the normal diploid fibroblast strains Flow 5000 and MRC-5.

The ts mutant tsK virus was used as it synthesizes only the IE proteins ICP0, 4, 22, 27 and 47 at the non-permissive temperature (Preston, 1979). This is due to a single base mutation in the ICP4 gene (Davison et al., 1984) resulting in the production of a thermolabile polypeptide at 38.5 °C. Any chromosome damage induced after infection at the non-permissive temperature would therefore be due either to the entry of the particle or the synthesis of IE proteins. Cells infected with 5 p.f.u./cell tsK at 3 and 6 h post-infection (Table 1) exhibited only an uncoiling of secondary constrictions most notably on chromosome 1, with 87% of metaphases showing uncoiling in the region 1q12-21 (Fig. 1c, d). This indicates that the first sign of damage by HSV is despiralization of specific pericentric regions on chromosomes 1, 9 and 16, and that the synthesis of β and γ proteins is not required for this phenomenon induced by tsK. It is probable that the chromatid breaks and pulverization observed after infection with HFEM are related to the synthesis of β and γ proteins which include viral polymerases and alkaline nucleases.

The ts mutant tsB7 at the non-permissive temperature enters the cell but is blocked at the nuclear pore due to a mutation in a late gene coding for a virion component (Knipe et al., 1981). The virus translates no detectable proteins and so enables one to determine whether entry of virus alone is sufficient for damage to occur, or whether limited viral transcription is required. Table 1 shows that a small percentage of cells exhibited structural damage after infection with tsB7 at 5 p.f.u./cell, 6 h after infection, although viral infection dramatically reduced the number of analysable metaphases. At 50 p.f.u./cell, 50% of the cells showed extensive damage exemplified by chromatid breaks, chromosome breaks and acentric fragments but no despiralization was apparent. It is probable that the large number of viral particles infecting the cell at this m.o.i. causes cellular damage due to activation of lysosomal enzymes (Allison & Paton, 1965) or to membrane perturbation and the release of active radicals.

Inactivated virus was used to ascertain whether the mere presence of the particle in the cell was an adequate requirement for induction of chromosomal damage. The inactivated virus prepared enters the cells with no viral polypeptide synthesis (SDS–PAGE, unpublished...
observations). Beta-propiolactone inactivates most viruses (LoGrippo, 1960) including HSV. Its mode of action is unclear but it is thought to alkylate the nucleic acid and prevent translation of the viral genome (Garlick & Avery, 1976), retaining its antigenicity yet with a reduction in infectivity. The virus titre was reduced by 10^7-fold using a 0.05% solution of β-propiolactone (Sigma) (Neff & Enders, 1968). Cells infected with β-propiolactone-inactivated HFEM at 5 p.f.u./cell showed 3% and 1% aberrations respectively, which probably represented the fraction of cells infected with residual wild-type virus. Problems may occur using β-propiolactone, as the inactivation procedure may affect the protein coat and reduce adsorption and entry. Short wavelength u.v. light has been used to inactive HSV (Ross et al., 1972), but inactivation is inefficient since production of infectious virus cannot be eliminated completely. Inactivation with psoralen and long wave-length u.v. (LW u.v.) was therefore used since viral DNA alone is damaged, not the viral capsid, and therefore infectivity is unaffected. Psoralen/LW u.v.-inactivated virus adsorbs to and penetrates the cell but viral transcription is prevented due to the irreversible binding of psoralen to viral DNA (Redfield et al., 1981). Ten μg psoralen (Sigma) was added to a 1.0 ml solution of virus, and the mixture was subjected to 365 nm u.v. (B-100A lamp, UV Products, Cambridge, U.K.) for 10 min, reducing the virus titre by 10^8-fold. In cells infected with psoralen/LW u.v.-inactivated virus no aberrations occurred at 3 h post-infection with a m.o.i. up to 50 p.f.u./cell. Six h after infection dicentric chromosomes were present in up to 50% of metaphases, but no despiralization of chromosomes 1, 9 and 16 occurred. Psoralen compounds have been shown to induce chromosome aberrations, and the dicentric chromosomes seen here are characteristic changes induced by the drug in first division cells (Hook et al., 1983). Our observations using inactivated virus agree with prior results obtained using HSV inactivated with short wave u.v. light (Rapp & Hsu, 1965; Waubke et al., 1968) or other methods of inactivation, e.g. heat (Hampar & Ellison, 1961), suggesting that the virus needs to undergo limited transcription before damage can occur. Since the methods of inactivation used in this report ensure entry of virus into the cell, we can confirm that one or more IE polypeptides need to be translated before HSV induces chromosomal aberrations.

Some specificity of chromosome damage in Chinese hamster cells after exposure to HSV-1 wild-type virus has been reported (Stich et al., 1964); in these cells there was preferential involvement of chromosomes 1 and X. In a recent study using human fibroblasts and lymphocytes (Mincheva et al., 1984), HSV-1 was shown to damage most frequently regions p32, p34, q21 and q32 on chromosome 1 and the short arm of chromosome 3. In our studies the region 1q21 underwent despiralization in the overwhelming majority of cells after exposure to the mutant tsK, and this was the earliest change in wild-type infection. All the chromosome sites involved in uncoiling after infection with adenovirus type 12, regions 17q21-22, 1p36, 1q21 and 1q42, are the sites of small nuclear (sn) RNA genes or SS RNA genes (Steffensen et al., 1976) and region 1q21 is the genomic locale of the class 1 U1 snRNA pseudogenes (Lindgren et al., 1985). It is possible that the uncoiling event reflects an increased transcription of these genes immediately following viral infection. Further studies are underway to investigate this possibility.

IE proteins are involved in transcriptional activation of delayed early promoters (Clements et al., 1977) and ICP0 and ICP4 will transactivate delayed early genes using expression vectors (O'Hare & Hayward, 1985). Recent work has shown ICP0 and ICP4 of HSV-1, and E1A of adenovirus to transactivate cellular as well as viral genes. These include heat shock and β-tubulin genes by E1A (Kao & Nevins, 1983; Green et al., 1983), and β-globin by HSV (Everett, 1984). It has been suggested that the transactivators induce a cellular transcription factor which binds to the promoter of the gene, enhancing its activity. The chromatin may be changing its conformation to accommodate the transcription factor, a change seen at the light microscope level as despiralization.

In summary, the present study shows that for chromosomal damage to occur following HSV-1 infection there must be synthesis of the IE polypeptides. Whether the damage is caused by one or more of the IE proteins or a virus-induced cellular protein is not clear. The initial damage is non-random, resulting in despiralization of chromosome 1q21 and to a lesser extent the pericentric regions of chromosomes 9 and 16.
REFERENCES


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