Induction of a Latent Herpes Simplex Virus from a Rat Tumour Initiated by Herpes Simplex Virus-transformed Cells

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SUMMARY

A rat tumour induced by cells transformed with the sheared DNA of herpes simplex virus (HSV) type 1 HFEM α (RE2A) was injected with the intertypic virus HSV-2 HG52 ts 1. Separate plaques, isolated from cocultivation of excised tumour tissue with susceptible cells, yielded virus the DNA of which had the restriction enzyme profile either of the injected HSV-2 virus or that of the HSV-1 virus, originally used to transform the cells. No evidence of in vivo recombination was detected. In Hooded Lister rats, HSV may have the ability to remain in a latent or non-replicating state in fibroblasts.

Rescue of the total transforming virus genome has been reported when SV40-transformed cells are fused with cells permissive for SV40 replication (Gerber, 1966). Similarly, but much less frequently, polyoma virus may also be rescued from polyoma-transformed cells (Chartrand et al., 1981; Fogel & Sachs, 1969). Furthermore, latent Epstein-Barr virus (EBV) can be induced from human lymphoid lines by treatment with halogenated deoxypyrimidines (Gerber, 1972).

We have previously reported that on homotypic or heterotypic superinfection of cells transformed by herpes simplex virus (HSV) we can rescue the virus originally used to transform such cells in addition to virus of recombinant genotype (Park et al., 1980). This finding suggested that in a small percentage of transformed cells the total virus genome used to transform the cells could be retained in a non-replicating or latent state.

We then asked the question whether such non-replicating virus could persist in a latent form in a tumour induced by these transformed cells in a host rat and whether such virus could be rescued by inoculation of the tumour in situ with heterotypic ts mutants of HSV.

Rat embryo cells, transformed by the sheared DNA of HSV-1 HFEM α, designated RE2A at passage 63 in culture were injected into the back of the neck of newborn rats (Hooded Lister) with 1 x 10^6 cells per animal and tumours allowed to develop over the following 12 to 18 months. This is an HFEM α-transformed cell line derived independently from that used by Park et al. (1980). As reported previously (Macnab, 1979) tumours have a long latent period in the inbred strain of rats (Hooded Lister) used for HSV transformation experiments in our laboratories.

A rat bearing a tumour induced by the RE2A (HSV-1 HFEM α) transformed cell line was injected with 5 x 10^6 p.f.u. of HSV-2 HG52 ts 1 virus (an heterotypic ts mutant) directly into the centre of the tumour mass. After 3 days the animal was sacrificed and the tumour tissue divided into two parts each of which was handled by separate workers. The tumour was finely minced and seeded onto subconfluent monolayers of BHK C13, Vero and chick embryo cells in 50 mm plastic tissue culture dishes (Nunc) at 31 °C, 37 °C and 38.5 °C. The permissive temperature for ts mutant virus replication is 31 °C and the non-permissive temperature for ts mutant virus replication is 38.5 °C. After 72 h incubation, plaques appeared on the susceptible cells at 31 °C. Isolated plaques were immediately picked, seeded onto subconfluent monolayers of BHK C13 cells in 50 mm plastic dishes and a virus plaque stock made for each isolate for further analysis.

The DNA of each plaque stock virus was extracted and subjected to restriction enzyme analysis by the micro method described by Lonsdale (1979) and used by Park et al. (1980). This...
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Fig. 1. Restriction enzyme profiles obtained after KpnI digestion of DNA labelled \textit{in vivo} with $^{32}$Porthophosphate from plaque isolates G, D, K, A, HSV-1, 17 syn$^+$ (lane 17), the original transforming virus HSV-1 HFEM a (lane a), and the superinfecting virus HSV-2 HG52 ts 1 (lane ts 1). G, D, K and A are representative of 32 plaques isolated after explantation of minced RE2A tumour tissue (a tumour derived from rat embryo cells transformed by the sheared DNA of HSV-1 HFEM a) into which HSV-2 HG52 ts 1 virus had been injected 3 days prior to explantation.

Analysis allows characterization of transforming virus, injected ts mutant and any recombinant molecules generated.

The restriction enzyme profile of the DNA (labelled \textit{in vivo} with $^{32}$Porthophosphate) from a total of 32 individual plaques arising on BHK C13 and Vero cells was analysed on agarose gels
after digestion with the enzymes KpnI, BamHI, HindIII and HpaI and subsequent autoradiography. The DNA from two of these 32 plaques had a restriction endonuclease profile indistinguishable from HSV-1 HFEM α, the virus originally used to transform the cells. The remaining 30 plaques had restriction endonuclease profiles indistinguishable from HSV-2 HG52 ts 1 the virus used to inject the tumour. Fig. 1 shows a KpnI digest of the DNA from four plaques (G, D, K and A). One syncytial plaque, G (Fig. 1), arising on Vero cells at 31 °C had a plaque morphology similar to HSV-1 HFEM α, and the KpnI restriction enzyme profile of DNA from this plaque was indistinguishable from that of HSV-1 HFEM α (lane labelled α in Fig. 1), the virus originally used to transform the cells. The DNA of plaques D, K and A (Fig. 1) have restriction enzyme profiles indistinguishable from HSV-2 HG52 ts 1, the virus used to inject the tumour. Fig. 1 represents the analysis for one-half of the tumour (cultured and analysed separately from the other half of the tumour). A second syncytial plaque whose DNA profile is also indistinguishable from that of HSV-1 HFEM α, arose from the other half of the tumour. This plaque was isolated in BHK C13 cells at 31 °C and had a similar plaque morphology to HSV-1 HFEM α. The other 30 isolates had the plaque morphology of HSV-2 HG52 ts 1.

The DNA of all 32 plaques isolated was further analysed on agarose gels after digestion with the enzymes BamHI, HindIII and HpaI but no plaques with DNA of a recombinant restriction endonuclease profile were detected. Only two isolates (each handled separately, one of which is shown in Fig. 1, plaque G) were found to have the restriction enzyme pattern of the original virus HSV-1 HFEM α used to transform the rat embryo cells. The DNA restriction enzyme profiles of the other 30 isolates were similar to HSV-2 HG52 ts 1. These experiments, carried out on two separate halves of a divided tumour sample, suggest that at least two parts (and at a minimum two separate cells) of the tumour contain the entire genome of HSV-1 HFEM α, the virus originally used to transform the RE2A cell lines (as detected by the limits of resolution of restriction enzyme analysis). Discrete plaques were not observed on chick embryo cells.

In addition, no in vivo recombination event between HSV-1 and HSV-2 was detected from analysis of the DNA of discrete plaques isolated on BHK C13 and Vero cells. However, had we detected recombinant molecules following restriction enzyme analysis this experiment could not prove whether such molecules arose as the result of an in vivo or an in vitro recombination event. This is in contrast to the results from superinfection of HSV-transformed cells where recombinant virus was detected (Park et al., 1980).

These experiments also suggest, as did our original findings (Park et al., 1980), that the total HSV genome may be retained in HSV-transformed and tumour fibroblast cells in a latent or non-replicating form after 63 passages in tissue culture and 18 months in the rat prior to development of the tumour. No evidence of skin eruption indicating herpetic infection was visible macroscopically in the tumour after injection and up to excision time. We have already shown that HSV titrated in HSV-transformed cells has a lower titre and smaller plaques than in normal permissive rat embryo or BHK C13 cells (Macnab, 1975). This finding may explain the lack of vesicles seen macroscopically in the injected rat tumour.

HSV-1 HFEM α replicating virus could not be isolated from other RE2A tumour explants or extracts disrupted by ultrasonication when cocultivated with susceptible cells. Nor, indeed, has treatment of explanted tumour cells with IUdR, BUdR or TPA succeeded in induction of replicating virus. These results are similar to those reported by Macnab (1974) where it was not possible to isolate virus from HSV-2-transformed cell lines.

This would suggest that in the inbred Hooded Lister rat, the fibroblast cell has the capacity to harbour latent non-replicating HSV genomes since free replicating virus has not been demonstrated in such transformed or tumour cells. This finding is similar to the result of latent virus detected in the guinea-pig footpad (Scriba, 1977; Fong & Scriba, 1980) and more recently in the mouse footpad (S. Al-Saadi & G. Clements, personal communication). The result is in contrast, however, to previous findings in rabbits and mice where HSV was latent only in nervous tissue.

At present, we do not understand what event(s) induces the replication of the latent virus in tumours as described in this report and previously for HSV-transformed cells (Park et al., 1980). It has been shown that expression of superinfecting virus gene products can switch on
transcription of resident virus genes in cell lines selectively transformed for the HSV thymidine kinase gene (Kit et al., 1978). We propose that viral gene products from superinfecting virus may induce replication of the latent virus which is present in transformed or tumour cells possibly by inducing or supplying enzymes required for DNA replication or by providing an origin of replication or necessary packaging sequences. Replication of the input virus could account for the high proportion of plaques isolated (30/32) whose DNA profile is indistinguishable from that of the virus, HSV-2 HG52 ts 1, used to inject the tumour. We cannot, however, discount the fact that only a few cells (perhaps as few as two) in the tumour contain the original transforming virus genome.

This technique of injection of an HSV-induced tumour with an heterotypic HSV virus has unambiguously allowed us to detect HSV DNA in tumour cells where it would not be possible to detect such HSV DNA by Southern blot analysis.

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REFERENCES


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