Expression of genes for the Epstein–Barr virus small RNAs EBER-1 and EBER-2 in Daudi Burkitt’s lymphoma cells: effects of interferon treatment

Paul A. Clarke,† Nigel A. Sharp2 and Michael J. Clemens1*

1Division of Biochemistry, Department of Cellular and Molecular Sciences, St George’s Hospital Medical School, Cranmer Terrace, London SW17 0RE and 2Department of Cell Biology, Wellcome Research Laboratories, Beckenham, Kent, U.K.

The relative levels, rates of synthesis and stabilities of the abundant Epstein–Barr virus (EBV)-encoded small RNAs, EBER-1 and EBER-2, were examined in Daudi Burkitt’s lymphoma cells. Although both RNAs are transcribed at approximately equal rates, the steady-state level of EBER-1 is at least 10-fold greater than that of EBER-2. This is shown to be due to a much faster rate of turnover of EBER-2. In the presence of actinomycin D, the half-lives of EBER-1 and EBER-2 are 8 to 9 h and 0.75 h, respectively. Following treatment of the cells with human interferon (IFN) α the transcription of both RNAs is strongly inhibited. However, the level of EBER-1 increases up to twofold, indicating a further stabilization of this RNA. In IFN-treated cells, EBER-2 accumulates in the form of truncated products. Nuclease protection experiments indicated that this is due to a post-transcriptional modification of the 3’ end of the molecule. These data show that the effects of IFN treatment on the expression of these two viral gene products are very complex in cells latently infected with EBV.

Introduction

Epstein–Barr virus (EBV) is a lymphotropic human herpesvirus that can immortalize B lymphocytes in vitro, giving rise to lymphoblastoid cell lines. In the majority of such cells the virus enters a latent phase in which gene expression is limited to a maximum of six nuclear antigens (EBNA 1 to 6) and up to three membrane proteins (Kieff & Liebowitz, 1990). In addition, two small untranslated, non-polyadenylated viral RNA species, EBER-1 and EBER-2, accumulate to high levels (Lerner et al., 1981; Rosa et al., 1981; Arrand & Rymo, 1982; Rooney et al., 1989; Alferi et al., 1991). In Burkitt’s lymphomas an even more restricted pattern of EBV gene expression is often observed, with only EBNA-1 and the EBERs being detectable (Gregory et al., 1990). The EBERs are the most abundant of all EBV-encoded transcripts and are present in the majority of EBV-positive cells, with the exception of oral hairy leukoplakia lesions in AIDS patients (Gilligan et al., 1990).

The function of these RNAs in vivo remains unclear because recombinant virus lacking both the EBER-1 and EBER-2 genes is able to immortalize B cells in tissue culture and can replicate in an apparently normal fashion (Swaminathan et al., 1991). Earlier in situ hybridization studies suggested that the EBERs may be localized predominantly in the cell nucleus (Howe & Steitz, 1986). However, the EBERs were originally identified as being associated with polysomes (Rymo, 1979) and recently high resolution confocal laser scanning microscopy has produced unambiguous evidence for the presence of both the EBERs in the cytoplasm of Raji Burkitt’s lymphoma cells (Schwemmle et al., 1992). The latter finding is consistent with biochemical and genetic evidence that EBER-1 and EBER-2 may have a role in translational control. The EBERs have been shown to complement a functionally analogous small RNA, the VA1 RNA of adenovirus, allowing the growth of otherwise defective mutants of this virus (Bhat & Thimmappaya, 1985). In vitro the EBERs mimic the behaviour of VA1 RNA, binding to an interferon (IFN)-induced protein kinase known as the dsRNA-activated inhibitor of protein synthesis (DAI or p68) (Clarke et al., 1991). This interaction prevents the autophosphorylation and activation of DAI (reviewed in Mathews & Shenk, 1991). In its activated form DAI inhibits initiation of translation by phosphorylating the α subunit of polypeptide chain initiation factor eIF-2.

† Present address: Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, New York 11724, U.S.A.
(Levin et al., 1980; Siekierka et al., 1985). Both EBER-1 and EBER-2 inhibit the activation of DAI and thus prevent the inhibition of protein synthesis by dsRNA in vitro (Clarke et al., 1990a, 1991; T. Sharp et al., unpublished results). Since DAI activity constitutes one of the mechanisms by which IFNs exert their antiviral effects, it is possible that the EBERs play a role in protecting EBV from these effects during the establishment of latent infections. However, Swaminathan et al. (1992) were unable to find any difference in sensitivity to IFN between EBER-positive and EBER-negative EBV-infected cell lines.

The steady-state level of EBER-1 is much higher than that of EBER-2 in most EBV-positive cell types, although the anomalous migration of these RNAs on denaturing polyacrylamide gels, owing to their extensive secondary structure, can cause difficulties in correctly distinguishing the two RNAs. The basis for the differential expression of the two species has not been established previously. The genes encoding the EBERs are located in tandem in the EBV genome (Rymo, 1979; Arrand & Rymo, 1982) and are transcribed by the cellular RNA polymerase III. They contain characteristic elements of the promoter for RNA polymerase III; in addition, the efficient transcription of the EBER genes requires upstream elements that are usually associated with sequences transcribed by RNA polymerase II (Howe & Shu, 1989).

The interaction of the EBERs with a component of one of the IFN-induced antiviral pathways, together with an earlier observation that EBER-1 expression may be up-regulated by IFN-α treatment of the Daudi Burkitt's lymphoma cell line (Clarke et al., 1990b), have led us to examine in more detail the relationship between IFN-α and the EBERs. In this paper we describe effects on the steady-state levels of EBER-1 and -2, and on the transcription and stability of these RNA species following IFN-α treatment of Daudi cells. We have also investigated a post-transcriptional modification of EBER-2 which occurs following IFN-α treatment.

**Methods**

**Cell culture and IFN-α treatment.** Cells were propagated as stationary suspension cultures at 37°C in a 5% CO₂ atmosphere. Growth medium was RPMI 1640 (Gibco) supplemented with 10% heat-inactivated foetal calf serum (Imperial Laboratories) or Nu-serum IV (Flow Laboratories).

Exponentially growing cells at a density of 2 × 10⁵ to 3 × 10⁶/ml were incubated with or without human lymphoblastoid IFN (Wellcome Laboratories) at concentrations up to 100 units/ml for various lengths of time. The IFN preparation, which is a mixture of IFN-α species, had a specific activity of greater than 10⁶ units/mg. Such treatment results in the inhibition of both cell growth and [³H]thymidine incorporation (Gewert et al., 1981).

**Cytoplasmic RNA extraction.** Cytoplasmic RNA was prepared as described by Bodescot et al. (1984) with modifications. Cells (1 × 10⁸ to 10 × 10⁸) were pelleted by centrifugation at 150 g for 10 min at 4°C and washed twice in ice-cold PBS (140 mM-NaCl, 2.7 mM-KCl, 8 mM-Na₂HPO₄, 1.5 mM-K₂HPO₄). The final cell pellet was resuspended at a concentration of 10⁶ cells/ml in NTM (150 mM-NaCl, 10 mM-Tris-HCl pH 7.8, 5 mM-MgCl₂). Vanadyl ribonucleoside complex (Gibco-BRL) was added to the cell suspension to a concentration of 11 mM, followed by 0.1 volumes of 3% NP40 in NTM, and the suspension was mixed very gently. After 10 min on ice, nuclei were pelleted at 1000 g for 10 min at 4°C. The cytoplasmic fraction was mixed with an equal volume of 20 mM-Tris-HCl pH 7.6, 2 mM-EDTA, 1% SDS and extracted twice with phenol/chloroform and once with chloroform. Any residual vanadyl ribonucleoside complex at the end of the extractions was removed by adding EDTA to a final concentration of 10 mM and leaving the solution on ice for 15 min. The RNA was then ethanol-precipitated, dissolved in 2 ml of water and redissolved with ethanol.

**Northern blotting.** Small RNAs (< 1 kb) were resolved by electrophoresis on denaturing 10% polyacrylamide gels in the presence of 7 M-urea in 90 mM-Tris-borate, 2 mM-EDTA, pH 8.3. RNA samples (2 to 5 µg) were heated at 65°C for 10 min before loading. Electrophoresis was carried out at 20 to 25 V/cm.

RNAs were transferred to nylon membranes by electroblotting as described by Murthy et al. (1986). The gel was equilibrated for 30 min in 10 mM-Tris-acetate pH 7.8, 5 mM-sodium acetate, 0.5 mM-EDTA, which also served to remove the urea, and electroblotting was carried out at 1 V/cm for 1 h and then at 4 V/cm for 2 h. At the end of the transfer process the RNA was covalently linked to the membrane by u.v. irradiation. RNAs were detected by hybridization with randomly primed radiolabelled DNA probes, followed by autoradiography (Sambrook et al., 1989).

**Nuclear run-on analysis.** The method of Einat et al. (1985) was used to isolate transcriptionally active nuclei from Daudi cells. Cells (10⁹) were centrifuged and washed twice in ice-cold PBS, resuspended in 40 ml of hypotonic buffer (10 mM-HEPES, K⁺ pH 7.6, 5 mM-MgCl₂, 25 mM-KCl, 0.1 mM-EDTA, 1 mM-DTT) and lysed by 15 strokes of a Dounce homogenizer. The nuclei were pelleted by centrifugation at 800 g for 15 min at 4°C, resuspended in 3 ml of hypotonic buffer, and loaded on to 8 ml of hypotonic buffer containing 25% glycerol and again pelleted at 2000 g for 15 min at 4°C. The nuclei were respersed to a concentration of 10⁶ nuclei/ml in hypotonic buffer/25% glycerol.

Aliquots (200 µl) of equal concentrations of isolated nuclei were incubated in a final volume of 500 µl in the presence of 30 mM-HEPES, K⁺ pH 7.5, 160 mM-KCl, 8 mM-MgCl₂, 0.5 mM-EDTA, 0.07 mM-EDTA, 5 mM-DTT, 10% glycerol, 0.4 mM each of GTP, CTP and ATP, 25 µM-UTP and 250 µCi [α-³²P]UTP or 50 µCi [³H]UTP at 25°C for 30 to 60 min. Labelled nuclear RNA was isolated by the LiCl/urea method (Auffray & Rougeon, 1980) with modifications. Nuclei were pelleted at 150 g for 7 min. The supernatant was passed through a Sephadex G-50 spin column and the excluded fraction (containing about 5% of the total labelled RNA) was collected and stored at -20°C. The nuclei were resuspended in 0.5 ml PBS and mixed with 5 ml of 3.3 M-LiCl, 6 M-urea. The resulting viscos le solution was passed through a 21 gauge needle several times to shear the chromosomal DNA, and left overnight at 4°C. Nuclear RNA was pelleted by centrifugation at 14000 g for 30 min and resuspended in 200 µl of 10 mM-Tris-HCl pH 7.6, 1 mM-EDTA, 0.5% SDS. This was then combined with the flow-through from the G-50 column. This procedure efficiently recovers the EBERs (N. A. Sharp, unpublished data).

Incorporation of total label was determined by TCA precipitation. The labelled RNA was used to probe Southern blots of appropriate
Results

IFN-induced changes in EBER-1 and EBER-2 steady-state levels

The relative levels of EBER-1 and EBER-2, and the effects of IFN-α treatment on these small RNAs, were investigated by Northern blot analysis of cytoplasmic RNA. In Daudi cells, as in other Burkitt's lymphoma and lymphoblastoid cell lines, EBER-1 is much more abundant than EBER-2 (Fig. 1 e). Use of EBER-1 and EBER-2-specific probes confirmed that these two small RNAs migrate anomalously on urea-polyacrylamide gels, with the higher M, EBER-2 migrating faster than EBER-1. Fig. 1 also shows the effects of IFN-α treatment on these small RNAs. As a control the level of U1 snRNA was also measured. The latter is unaffected by IFN-α treatment (b and d), whereas the level of EBER-1 is increased by around 1-6-fold during IFN-α treatment (a). This effect, although relatively small, is reproducible. In several experiments in our laboratory the increase in EBER-1 due to IFN-α treatment varied within the range of 1·4- to twofold. Treatment with 100 units/ml IFN-α for 1 day was sufficient to cause this effect (data not shown). The level of EBER-2 is altered by IFN-α treatment in a complex manner. There is some decrease in the steady-state level of this RNA; however, more notable is the appearance of two smaller doublets after IFN-α treatment (Fig. 1 c).

Transcription of EBER genes

The relative transcriptional activities of the EBER genes in Daudi cells and the effects of IFN-α on EBER transcription were investigated by a nuclear run-on assay. Characterization of the transcriptional behaviour of nuclei from control and IFN-α-treated cells showed little difference in overall activity or response to inhibitors (data not shown). Fig. 2 shows that in control Daudi cells the rate of transcription of the EBER-2 gene was very similar to that of the EBER-1 gene. Allowing
for the different numbers of U residues in EBER-1 and EBER-2 we calculate that the ratio of EBER-2 to EBER-1 transcription is approximately 1.5:1. IFN-α treatment of Daudi cells caused a decrease in transcription of both EBER genes of approximately 65%. In contrast, the transcription of another EBV gene, for the nuclear antigen EBNA-1, was not down-regulated by IFN-α and may even have been enhanced (Fig. 2). This indicates that the decrease in the number of EBER transcripts in newly synthesized RNA from nuclei of IFN treated cells was not due to a non-specific change in RNA labelling or hybridization.

Stability of EBER-1 and EBER-2 in control and IFN-treated Daudi cells

The relative steady-state levels of EBER-1 and EBER-2 in control cells, and the effects of IFN-α treatment on these levels, cannot be accounted for by differences in the rates of transcription of the genes for these two small RNAs. To investigate post-transcriptional mechanisms that might explain these discrepancies, the stabilities of the EBERs were measured by Northern blotting of cytoplasmic RNA isolated from cells at different times after treatment with actinomycin D. Cells were incubated with or without 100 units/ml IFN-α for 24 h prior to the inhibition of further RNA synthesis. Fig. 3 shows that under these conditions EBER-1 was relatively stable in both control and IFN-α-treated cells. Laser densitometry of several different exposures of the autoradiographs indicated a half-life of at least 8 to 9 h for EBER-1; however, a semi-log plot of band intensity versus time of actinomycin treatment indicated some heterogeneity in the turnover kinetics of this RNA, with a substantial fraction having a longer half-life. IFN-α treatment had no measurable effect on the turnover of EBER-1, although given the stability of this RNA and the nature of its decay kinetics it is doubtful whether any changes in half-life would be readily observed.

Fig. 3 also shows that EBER-2 was much less stable than EBER-1 in both control and IFN-α-treated cells. Densitometry gave a half-life of approximately 45 min. IFN-α treatment for 24 h again resulted in the appearance of bands below the main EBER-2 band, as noted previously (Fig. 1). The data indicated that these additional bands are more stable than full-size EBER-2 and, because they appear even when transcription is blocked with actinomycin D, they must correspond to post-transcriptionally modified forms of EBER-2. These products were specific to RNA samples isolated from IFN-treated cells and were not observed when the growth of Daudi cells was inhibited by other means such as phorbol ester treatment or growth to high density (N. A. Sharp, unpublished data). Treatment with IFN-α for only 3 h did not give rise to the faster migrating forms of EBER-2 (not shown).

Analysis of the IFN-induced modification of EBER-2

RNase protection analysis was used to identify the nature of the post-transcriptional shortening of EBER-2 observed in IFN-α-treated cells. Fig. 4 shows the results obtained using a probe specific for the 3' end of EBER-2. No protection of the probe was observed in the absence of added cytoplasmic RNA (not shown), or in the presence of RNA from EBV-negative Ramos cells (lane 1), indicating that the probe could not protect itself against digestion. A single protected fragment of the predicted size (98 nucleotides) was observed in the presence of control Daudi cell cytoplasmic RNA, whereas in the presence of RNA from cells treated with 100 units/ml IFN-α for 1 or 2 days (with or without subsequent actinomycin D treatment) both this fragment and a smaller protected fragment were observed. This
the EBER-2 gene was constructed by ligating a 286 bp EcoRI-AgoI fragment of plasmid pJJJ-2 into the transcription vector pGEM-2. This construct was digested with HhaI and a 683 bp fragment containing the 3' end of plasmid pJJJ-2 into the transcription vector pGEM-2. This purified and used in RNase protection analysis of cytoplasmic RNA as antisense RNA (296 nucleotides). The labelled transcript was gel-purified and transcribed with SP6 polymerase in the presence of [α-32P]UTP to generate a labelled RNA probe (98 nucleotides). The size of the RNA probe protected by the 3' end of intact EBER-2 (indicated by the arrow) is 98 nucleotides.

Cytoplasmic RNA was from the following sources. Lane 1, Ramos cells (EBV-negative); lane 2, control Daudi cells; lane 3, IFN-α-treated Daudi cells (24 h, 100 units/ml); lane 4, IFN-α-treated Daudi cells (3 h, 100 units/ml); lane 5, control Daudi cells; lane 6, IFN-α-treated Daudi cells (48 h, 100 units/ml). The cells used for the RNA preparations in lanes 2 to 4 were incubated with actinomycin D (5 μg/ml) for 6 h prior to harvesting as part of the experiment designed to measure the stability of EBER-2 in the absence of further transcription (see Fig. 3). The samples in lanes 5 and 6 are from a separate experiment and contained less RNA. (b) A longer exposure of lanes 2 to 6.

Fig. 4. RNase protection analysis of the 3' terminus of EBER-2 in control and IFN-treated Daudi cells. A plasmid containing the 3' end of the EBER-2 gene was constructed by ligating a 286 bp EcoRI-AgoI fragment of plasmid pJJJ-2 into the transcription vector pGEM-2. This construct was digested with HhaI and a 683 bp fragment containing the 3' end of intact EBER-2 (indicated by the arrow) is 98 nucleotides. The labelled transcript was gel-purified and used in RNase protection analysis of cytoplasmic RNA as described in Methods. The size of the RNA probe protected by the 3' end of intact EBER-2 (indicated by the arrow) is 98 nucleotides. Cytoplasmic RNA was from the following sources. Lane 1, Ramos cells (EBV-negative); lane 2, control Daudi cells; lane 3, IFN-α-treated Daudi cells (24 h, 100 units/ml); lane 4, IFN-α-treated Daudi cells (3 h, 100 units/ml); lane 5, control Daudi cells; lane 6, IFN-α-treated Daudi cells (48 h, 100 units/ml). The cells used for the RNA preparations in lanes 2 to 4 were incubated with actinomycin D (5 μg/ml) for 6 h prior to harvesting as part of the experiment designed to measure the stability of EBER-2 in the absence of further transcription (see Fig. 3). The samples in lanes 5 and 6 are from a separate experiment and contained less RNA. (b) A longer exposure of lanes 2 to 6.

indicates that a fraction of EBER-2 was modified at the 3' end during IFN-α treatment. Taken together with the Northern blotting data (Fig. 1 and 3) the nuclease protection results established that the shortening is from the extreme 3' end of the molecule (as opposed to endonucleolytic cleavage of the central part of EBER-2). The difference in sizes of the protected fragments is approximately four nucleotides, indicating loss of the terminal oligo(U) sequence (Glickman et al., 1988). RNA from cells treated for only 3 h with IFN did not give rise to the smaller protected fragment (Fig. 4). Although in some experiments more than one truncated form of EBER-2 was seen on Northern blots of RNA from cells treated for 24 or 48 h with IFN-α (e.g. Fig. 1), only one predominant smaller species was detected in the RNase protection experiments. This discrepancy may be due to variable degrees of denaturation of the RNA on urea gels.

Discussion

In this study we examined the relative synthesis rates of the EBERs and the subsequent fate of these RNAs in the cytoplasm of Daudi cells. Although one report has suggested that the EBERs have a nuclear location (Howe & Steitz, 1986) more recent in situ hybridization studies (Schwemmlle et al., 1992) have indicated that a substantial fraction of both RNAs is found in the cytoplasm. Our experiments did not address the question of whether the EBERs in the nucleus behave similarly to the cytoplasmic EBERs with respect to stability or post-transcriptional modification.

The nuclear run-on experiments (Fig. 2) indicate a slightly higher rate of transcription for EBER-2 than for EBER-1. In view of the very different steady-state levels of the two RNAs, EBER-1 would therefore be predicted to be far more stable than EBER-2. We have confirmed this experimentally, estimating the half-lives of these small RNAs to be 8 to 9 h and 0.75 h, respectively. If EBER-1 and EBER-2 were synthesized at equal rates it can be calculated from these figures that the steady-state level of EBER-1 should exceed that of EBER-2 by about 10- to 12-fold. The good agreement between this prediction and the observed relative abundance of EBER-1 and EBER-2 adds credence to the measured rates of transcription in Daudi cells. The relative levels of expression of the EBERs have been previously studied by labelling experiments. Immunoprecipitation of La antigen–EBER complexes containing RNA labelled for 24 h in vivo quantitatively recovered both labelled RNAs, with equal amounts of radioactivity in each (Howe & Shu, 1988; Lerner et al., 1981). However, in view of the stability of EBER-1, such a protocol would probably underestimate the total amount of EBER-1 in the cell. Pre-existing unlabelled EBER-1 also may not necessarily be associated with the La antigen. It is unclear whether EBER molecules bound to proteins would in any case have the same rate of turnover as free RNA.

We have observed a number of effects of IFN-α treatment on EBER gene expression, although because our studies have been confined to Daudi cells it is not yet clear to what extent these effects may be seen in other EBV-positive cell lines. The synthesis of both EBER-1 and EBER-2 is strongly inhibited in IFN-α-treated cells. IFNs have been shown to regulate gene expression via the interaction of trans-acting factors with response elements upstream of genes transcribed by RNA polymerase II (Williams, 1991). However, the EBERs
are RNA polymerase III transcripts and there is no precedent for such gene products being regulated by IFN at the transcriptional level. It is possible either that regulatory sequences in the EBER genes bind an IFN-α-induced transcriptional repressor, or that constitutive factors that bind to EBER control elements are modified in some manner by an IFN-α-induced mechanism. The factor affected by IFN-α may regulate a specific subset of RNA polymerase III genes because the nuclear run-on experiments show a 65% decrease in EBER transcription but we have observed little effect on total RNA polymerase III activity (P. A. Clarke, unpublished data).

Comparisons of the nuclear run-on results with the data from Northern blotting indicate that IFN-α must also regulate EBER expression at the post-transcriptional level. As transcription is strongly decreased following IFN-α treatment the stability of EBER-1 must increase significantly to account for the RNA levels observed. The expected increase in the half-life of EBER-1 of more than twofold was not observed in the actinomycin decay experiment shown in Fig. 3. However, since the stabilization of EBER-1 may involve association with an IFN-induced protein or RNA, the use of actinomycin as a general transcriptional inhibitor would block the further synthesis of mRNAs encoding factors that could protect EBER-1 (such factors may themselves be relatively short-lived). In addition, owing to the relatively long half-life of EBER-1 in control cells, the predicted stabilization of this RNA may be difficult to observe under the conditions used.

It is of interest that IFN-α treatment has opposite effects on the synthesis and stability of different EBV transcripts, i.e. EBER-1 and EBNA-1, in Daudi cells. We have reported previously that the level of EBNA-1 mRNA is decreased in IFN-α-treated Daudi cells (Clarke et al., 1990b); the data in this paper suggest that this may be due to RNA destabilization because the transcription rate of this gene is not impaired.

It is not known whether the changes in the post-transcriptional regulation of the EBERs may be due to the actions of IFN-induced proteins such as the DAI protein kinase or the 2′,5′ oligoadenylate synthetases (Pestka et al., 1987). DAI is induced within 6 to 9 h of IFN-α treatment (Meurs et al., 1990) and data from this laboratory have demonstrated interactions between DAI and both EBER-1 and EBER-2 (Clarke et al., 1990a, b; T. Sharp et al., unpublished results). 2′,5′ oligoadenylates regulate RNA turnover through the activation of a specific ribonuclease, RNase L (Clemens & Williams, 1978; Pestka et al., 1987). Although there is no evidence that the EBERs are substrates for RNase L, approximately four nucleotides are removed from the 3′ terminus of EBER-2 in IFN-α-treated cells, corresponding to the terminal U residues of the sequence CUAUUUUU. Since UU and UA dinucleotides in ssRNAs are known RNase L cleavage sites (Wreschner et al., 1981), the 3′ end of EBER-2 contains several potential sites for this enzyme. Further experiments will be required to determine whether RNase L is responsible for the post-transcriptional shortening of EBER-2.

We are grateful to Vivienne Tilleray and Michael Luckcombe for skilful technical assistance, to Drs John Arrand and Anne Goodeve for gifts of plasmids pJ1-1, pJ1-2, pJF and pK.R1, and to Wellcome Laboratories for the human lymphoblastoid cell IFN. This research was supported by grants from the Cancer Research Campaign, the Leukaemia Research Fund, the Wellcome Trust, the Gunnar Nilsson Cancer Research Trust and the Sylvia Reed Cancer Fund. P. A. C. was supported by a studentship from the Medical Research Council.

References


Effects of interferon on EBV small RNAs


