The transcription factor YY1 binds to negative regulatory elements in the human cytomegalovirus major immediate early enhancer/promoter and mediates repression in non-permissive cells

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ABSTRACT

We have previously shown that repression of human cytomegalovirus (HCMV) major immediate early (IE) gene expression in non-permissive human teratocarcinoma (T2) cells is associated with a number of nuclear factors which bind to the imperfect dyad symmetry located in the modulator region upstream of the major IE enhancer as well as to the 21 bp repeat elements within the enhancer. Differentiation of T2 cells with retinoic acid (RA) results in a decrease in binding of some of these nuclear factors to these sites and deletion of these specific binding sites from major IE promoter/reporter constructs results in increased IE promoter activity in normally non-permissive cells. In this study, we demonstrate that the transcription factor YY1, which can negatively regulate the adeno-associated virus P5 promoter, directly binds to both the imperfect dyad symmetry and the 21 bp repeat elements in the HCMV major IE promoter/regulatory region and mediates repression of HCMV IE gene expression. This strongly suggests that YY1 plays an important role in regulating HCMV expression in non-permissive cells.

INTRODUCTION

HCMV, a member of herpesvirus family, is a ubiquitous human pathogen (1) which rarely causes disease in the immunocompetent host. As with all herpesviruses, a biological property of HCMV is its ability to establish life long persistence after primary infection. Reactivation of the virus, which is often associated with immunosuppression, can then result in severe and often fatal disease. However, the molecular mechanisms of latency and reactivation are still unclear. During productive infection, HCMV expression undergoes three phases of gene expression, immediate early (IE), early (E) and late (L). IE gene expression is known to be essential for regulation of both early and late phases of HCMV gene expression (2) and therefore plays an essential role

in productive infection. The human teratocarcinoma cell line NTeraT2D1 (T2) is non-permissive for HCMV infection which is due at least in part to a block of transcription in IE gene expression (3). However, differentiation of T2 cells with RA permits IE gene expression (3–6). Therefore, this cell line provides us an useful model system to study differentiation-specific cellular factors that regulate viral gene expression which may be involved in the control of latency.

The regulatory region of the HCMV major IE gene contains: a promoter which has a TATA motif for directing transcription using cellular RNA polymerase II (7, 2); a strong and complex enhancer which comprises 17 bp, 18 bp, 19 bp and 21 bp repeat elements (8–10); a cluster of nuclear factor 1 (NF1) binding sites (11, 12); and a differentiation dependent modulator sequence (13, 6). The 18 bp and 19 bp repeats have been extensively characterised and are believed to bind NF κ B and cyclic AMP-responsive element binding factors (CREB) respectively (14, 15). Little is known about the 17 bp repeat, but it can be deleted without negative effect on the strength of the promoter while the 18 bp and 19 bp repeats are functionally important elements for basal promoter activity (16). The NF1 binding sites are putative sites for the CCAAT box binding super family of proteins. We have previously shown that the 21 bp repeat elements within the enhancer and the imperfect dyad symmetry in the modulator region act as binding sites for a number of, so far, unidentified cellular factors which have been implicated in the negative regulation HCMV IE gene expression in non-permissive undifferentiated T2 cells (17, 18). Consistent with this, differentiation of these cells or deletion of the specific binding sites from IE promoter constructs permits IE gene expression (17, 18).

Here we report that the transcription factor YY1, also referred as UCRBP, NFE1 and δ (19–24), is one of the factors present in undifferentiated non-permissive T2 cells which specifically binds to the imperfect dyad symmetry and the 21 bp repeat element *in vitro*. YY1 has been shown to bind to the adeno-associated virus P5 promoter and implicated in both

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transcriptional activation and repression (23, 24). Similarly, UCRBP binds to a negative regulatory element in the upstream conserved region of the long terminal repeat (LTR) of Moloney murine leukaemia virus (MuLV) and also represses this LTR in undifferentiated F9 murine cells (20). Consistent with this we show that YY1, which is abundantly present in undifferentiated non-permissive T2 cells and absent in permissive differentiated T2 cells, mediates repression of HCMV IE gene expression but that this repression is dependent on additional differentiation-specific cellular factors. These data implicate the involvement of YY1 in the repression of HCMV IE gene expression in non-permissive cells and its role in control of HCMV latency is discussed.

MATERIALS AND METHODS

Cell lines

The undifferentiated teratocarcinoma cell line T2 (25) was maintained in DMEM supplemented with 10% FCS. Cells were split 1:3 every three days. Differentiation of T2 cells (T2RA) was induced by addition of 10^{-6} M alltrans retinoic acid for 5 days.

Plasmids, DNA transfections and CAT assays

The reporter plasmids pEScat and pIEP1cat have been previously described (17, 18, 26). In these plasmids the CAT reporter gene has been placed under the control of the full length HCMV (strain AD169) major IE promoter (comprising promoter sequences from -2100bp to +72bp) and the short IE promoter (comprising promoter sequences from -302bp to +72bp) which lacks the imperfect dyad symmetry and the 21 bp repeat elements. The plasmid SVYY1 was constructed by excising a YY1 EcoRI fragment from the plasmid pCMVYY1 (kindly provided by Thomas Shenk) containing the full length YY1 coding region and then cloning it into EcoRI digested pHK3 (Christian Hagemeyer and Tony Kouzarides, unpublished), placing YY1 under the control of the SV40 early promoter. The plasmid pSV2neo has been described previously (27). A HIS-YY1 plasmid (a gift of Thomas Shenk) allowed bacterial expression and purification of His-tagged YY1 protein as previously described (24).

For DNA co-transfection, approximately 5×10^6 cells were transfected with 100 ng of pEScat or pIEP1cat together with 1 μ g effector DNA by calcium phosphate precipitation. Cells were harvested 48 hours post-transfection and equivalent amounts of protein for T2 or T2RA extracts were assayed for CAT expression. Results shown are averages of at least 3 independent experiments.

Electrophoretic mobility shift assays (EMSAs)

Nuclear extracts (10 μ g) prepared essentially as described (18) or 200–400ng of bacterially expressed His-tagged fusion protein were assayed for their ability to retard approximately 5 ng of probe, labelled by filling in with 32 P-dCTP and Klenow. Binding reactions contained 5 μ g of polydIdC as non-specific competitor and were incubated for 30 min at room temperature with probe prior to loading on 8% polyacrylamide gels in $0.5 \times$ TBE. Where indicated, approximately a 10-fold, 100-fold or 200-fold molar excess of additional cold competitor was added to the binding reaction for 10 min prior to addition of probe. Below are the sequences of the 21 bp repeat element, the YY1 binding motif, the 5' half of the imperfect dyad symmetry, 3' half of the imperfect dyad symmetry, octamer motif and a mutated

YY1 binding motif. Nucleotides filled in by klenow are shown in the lower case.

The 21 bp element probe was:
 ACTTACGGTAAATGGCCCGCTGGCTgaccg
 tgaatGCCATTACCGGGCGGACCGACTGGC
 The YY1 motif probe was:
 GTTTTGCGACATTTTGCgacac
 caaaaCGCTGTAAACGCTGTG
 The 5' half of dyad symmetry probe was:
 AACTTTTGAAAAATGGCGATatcag
 tgaAAACCTTTTACCGCTATAGTC
 The 3' half of dyad symmetry probe was:
 GATTTTGGGCATACGgatatctg
 ctaaaaaccCGTATGCGCTATAGAC
 The octamer competitor was:
 GATCCTTAATAATTGTCATACCCTca
 cTAGGAATTATTAACGTATGGGAGT
 The mutant YY1 competitor was:
 GTTTTGCGAGTTGGCGAcac
 caa AACGCTCAACCGCTGTG

Western blot analysis

Approximately 10 μ g of nuclear extracts were separated on 10% gels by SDS-PAGE and transferred to Hybond-C nitrocellulose (Amersham). YY1 protein was detected by a YY1 monoclonal antibody (kindly provided by Thomas Shenk) with ECL reagents (Amersham) as described by the manufacturer.

Purification and labelling of GST fusion proteins

The plasmid pCMVYY1 was digested with NcoI, treated with Klenow to generate blunt ends, then digested with EcoRI to generate a fragment which could be subcloned into a SmaI/EcoRI deletion of pGEX.2TK, a glutathione-S-transferase fusion vector which contains a kinase domain permitting *in vitro* 32 P labelling of recombinant GST fusion protein (28). This generated the recombinant plasmid pGEX.2TK.YY1. The YY1 GST fusion proteins were prepared as previously described (28). For labelling, GST or GSTYY1 beads were resuspended in reaction buffer containing protein kinase and 32 P- γ -ATP and incubated at 4°C for 30 min (29). The beads were then washed five times with NETN buffer and resuspended in freshly prepared 20 mM glutathione in 100 mM Tris.HCl, pH 8.0, 120 mM NaCl, followed by incubating at 4°C for 10 min. Finally, the beads were spun down and the supernatant was carefully collected.

Far-western blot analysis

Approximately 10 μ g of nuclear extracts were separated by SDS-PAGE on 10% gels and transferred to nitrocellulose. The filters were treated in blocking buffer (1 $\times$ HBB, 5% dried milk, 1 mM DTT and 0.05% NP-40) overnight at 4°C. The filters were then denatured by two sequential washings in denaturing solution (1 $\times$ HBB, 6 M Guanidine-HCl and 1 mM DTT) for 10 min. each then once in denaturing solution diluted 1:1 with 1 $\times$ HBB for 10 min. Sequential dilution and washing was repeated until the filters had gone through 6 M, 6 M, 3 M, 1.5 M, 0.75 M, 0.375 M and 0.187 M Guanidine-HCl. The filters were then placed in 1 $\times$ HBB containing 1 mM DTT for 10 min, followed by incubating in 1 $\times$ HBB, 5% dried milk, 1 mM DTT, 0.05% NP-40 for 1 h then in 1 $\times$ HBB, 1% dried milk, 1 mM DTT, 0.05% NP-40 for 30 min. All steps were performed at 4°C. The filters were then incubated in hybridisation buffer (20 mM HEPES, pH 7.7, 75 mM KCl, 0.1 mM EDTA, 2.5 mM MgCl₂, 1% dried milk and 0.05% NP-40) containing GEX.2TK crude bacterial extracts with 32 P labelled protein probe overnight

at 4°C. Thereafter, the filters were washed in the hybridisation buffer without probe.

RESULTS

YY1 binds to the 5' half of imperfect dyad symmetry and the 21 bp repeat element

We have previously shown that the imperfect dyad symmetry in the HCMV major IE promoter modulator region as well as the 21 bp repeat elements in the HCMV major IE enhancer are negative regulatory elements for HCMV IE gene expression in undifferentiated non-permissive T2 cells (17, 18). The 21 bp repeat element forms a number of complexes upon electrophoretic mobility shift assays (EMSAs) with nuclear extracts prepared from undifferentiated T2 cells, which includes a complex we have previously termed MBF1 (17). Whilst the MBF1 complex is similar if not identical to that formed between the 3' half of HCMV imperfect dyad symmetry and T2 nuclear extracts (17, 18), the other faster migrating complexes observed between the 21 bp repeat and T2 nuclear extracts are similar to those observed between the 5' half of the imperfect dyad symmetry and T2 nuclear extracts (17, 18). This previously suggested to us that the T2 nuclear factors that interact with the 21 bp repeat are similar to those factors that bind to the imperfect dyad symmetry (both 3' and 5') as a whole (17, 18). We have also previously noted that the MBF1 complex dramatically decreases to almost undetectable levels with nuclear extracts prepared from differentiated permissive T2 cells and have suggested that this factor/s may be a candidate for a differentiation-specific repressor of HCMV IE expression (17). Analysis of the 5' half of imperfect dyad symmetry and the 21 bp repeat elements reveal that both sequences have good homology to the binding motif for a recently identified transcription factor, YY1 (Fig. 1), which has been shown to act as both a positive and negative regulator of a number of genes.

To test whether the factor(s) present in T2 nuclear extracts that interact with the 5' half of the dyad symmetry and/or the 21 bp repeat elements are YY1-like, we performed cold competition EMSAs with T2 nuclear extracts using the 21 bp repeat element or the 5' half of imperfect dyad symmetry as probes in the presence or absence of cold YY1 binding motif. As shown in Fig. 2A, all the complexes formed between the 21 bp repeat element probe and T2 nuclear extracts are competed for by the cold 21 bp repeat element as expected, but a specific subset of these complexes are also competed for by cold YY1 competitor. Interestingly, this does not appear to include the previously defined MBF1 complex but only the faster migrating complexes (which we will now term MBF4) which we have not previously studied in any detail. Similarly, cold YY1 competitor can also

compete for the complexes formed between the 5' half of dyad symmetry and T2 nuclear extracts (Fig. 2B), but only weakly with the complex formed with the 3' half of dyad symmetry (Fig. 2C). Consequently, these experiments show that the complexes formed with the 5' half of dyad symmetry and the 21 bp repeat elements contain YY1-like factors. The observation that the MBF1 complex formed between the 21 bp repeat element and T2 nuclear extracts can be competed for only by the cold 3' half of the dyad symmetry (Fig. 2A lane 6), whilst the MBF4 complexes can only be competed for by the 5' half of the dyad symmetry (Fig. 2A lane 5), also confirms our previous results which have suggested that the factors that bind to the whole dyad symmetry (both the 5' and 3' halves) are similar if not identical to those which bind to the 21 bp repeat elements (17, 18).

To investigate whether the YY1 binding motif has a similar mobility shift pattern as the imperfect dyad symmetry and the 21 bp repeat element with nuclear extracts, we carried out EMSAs with undifferentiated T2 and differentiated T2RA nuclear extracts using the YY1 binding motif as a probe. As seen in Fig. 3A, the YY1 probe forms two major DNA-protein complexes with T2 nuclear extracts which are similar to the MBF4 complexes formed with the 21 bp repeat probe or 5' dyad symmetry probe. These two complexes can be competed for by cold YY1 binding motif, 5' half of dyad symmetry or 21 bp repeat element, but not by cold 3' half of dyad symmetry. As expected, a mutant oligonucleotide mutated at sites specific for interaction with YY1 (24) did not compete for the MBF4 complex formed between T2 extracts and a YY1 binding site, 21 bp repeat or 5' dyad symmetry probes (Fig. 3B).

Whilst it was not apparent to us in our original observations, it has now become clear that, like MBF1, the MBF4 complexes are also differentiation specific and disappear upon RA-induced differentiation (see Fig. 4 lane 2 and also data in references 17 and 18).

To confirm that YY1 binds directly to the 5' half of dyad symmetry and the 21 bp repeat element, purified bacterially expressed His-tagged YY1 fusion protein was used in EMSAs with the 5' half of dyad symmetry and the 21 bp repeat element

Figure 1. DNA binding sequence comparisons. Sequences with similarity to each other are underlined.

Figure 2. Competition EMSAs on the 21 bp repeat element (A), the 5' half of dyad symmetry (B) or the 3' half of dyad symmetry (C) using undifferentiated T2 nuclear extracts. Shifts were competed with an additional 200-fold molar excess each of cold non-specific poly dIdC (0), 21 bp repeat element (21), 5' half of dyad symmetry (5'), 3' half of dyad symmetry (3') or YY1 binding motif (YY1). Binding assays with no nuclear extracts are shown in lane 1.

Figure 3. (A) Competition EMSAs on the YY1 binding motif using undifferentiated T2 nuclear extracts. Shifts were competed with an additional 200-fold molar excess each of cold non-specific poly dIdC (0), YY1 binding motif (YY1), 5' half of dyad symmetry (5'), 3' half of dyad symmetry (3') or 21 bp repeat element (21). Binding assays with no nuclear extracts are shown in lane 1. (B) Competition EMSAs on the 21 bp repeat, YY1 motif and 5' half of the dyad symmetry using undifferentiated T2 nuclear extracts. Shifts were competed with an additional 200-fold molar excess of cold poly dIdC (0), YY1 binding motif (YY1) or mutant YY1 binding motif (YY1mut).

as probes. As shown in Fig. 5A, bacterially expressed YY1 binds very strongly to the 5' half of dyad symmetry probe as well as the 21bp repeat but not the 3' half of the dyad symmetry (note that half the amount of recombinant YY1 was used in the binding assay for the 5' half of the dyad symmetry). This binding is specific as these YY1 complexes can be competed for by cold 5' half of dyad symmetry as well as a YY1 binding motif but not by cold octamer binding motif or a mutant YY1 binding motif (Fig. 5B). The cold 21 bp repeat element also specifically abolishes this binding, confirming that YY1 also binds to the 21 bp repeat element (Fig. 5B) and, as expected, binding of the 21 bp repeat element to bacterially expressed YY1 is competed for specifically by cold YY1 competitor (Fig. 5C). These results directly confirm that the 5' half of dyad symmetry and the 21 bp repeat elements both contain functional YY1 binding sites.

YY1 decreases upon differentiation of T2 cells with RA

As EMSAs showed that the YY1-specific complexes present in undifferentiated cells disappeared upon differentiation with RA, the levels of YY1 protein in T2 cells before and after retinoic acid treatment was analysed by Western blot using a YY1 specific antibody (Fig. 6). A differentiation-specific decrease in YY1 protein was observed, consistent with a previously observed decrease in YY1 RNA during long term differentiation of murine F9 embryonal carcinoma cells with RA (19). These results demonstrate that YY1 expression decreases upon RA-induced differentiation of T2 cells to a cell type which permits HCMV IE gene expression.

YY1 mediates repression of HCMV IE gene expression

To test whether YY1 does play any role in the negative regulation of HCMV IE gene expression, co-transfections of IE reporter plasmids in the presence of YY1 expressing plasmid were carried out. pEScat and pIEP1cat were transiently introduced into undifferentiated and differentiated T2 cells in the presence or

Figure 4. EMSAs of the YY1 binding motif (lane 1 and 2), the 21 bp repeat element (lane 3 and 4), the 5' half of dyad symmetry (lane 5 and 6) or the 3' half of dyad symmetry (lane 7 and 8) using undifferentiated (-RA) and differentiated (+RA) nuclear extracts prepared from T2 cells. Note that MBF2 and MBF3, complexes which we have previously defined (17, 18) and whose presence do not appear to be correlated with repression of major IE gene expression, are not seen in these low exposure autoradiographs.

absence of pSV-YY1, and the CAT activities were measured. As shown in Fig. 7, pSV-YY1 strongly decreases CAT activity in undifferentiated T2 cells driven by the full length IE promoter/reporter, pEScat, which contains the dyad symmetry and 21 bp repeat element binding sites for YY1 but not by the short IE promoter/reporter, pIEP1cat, which lacks YY1 binding motifs. However, the SV40 early promoter alone (pHK3) or

Figure 5. (A) EMSAs on the YY1 binding motif (1), 21 bp element (2), 3' half (3) or 5' half (4) of the dyad symmetry with 400ng (tracks 1, 2 and 3) or 200ng (track 4) of purified recombinant His-tagged YY1 protein. (B) EMSAs on the 5' half of dyad symmetry with 200ng of purified His-tagged YY1 in the presence of an additional 10-fold molar excess (lanes 3, 5, 7, 9, 11 and 13) or 100-fold molar excess (lanes 4, 6, 8, 10, 12 and 14) of each of the following cold non-specific competitor; polydIdC (0), 5' half of dyad symmetry (5'), YY1 binding motif (YY1), 21 bp repeat element (21), 3' half of dyad symmetry (3'), octamer binding motif (oct) (40) or mutant YY1 (YY1M) binding motif. Lane 1 contains no recombinant protein. (C) EMSAs on the 21 bp repeat element with 400ng of His-tagged YY1. Shifts were competed with an additional 100-fold molar excess of poly dIdC (0), 21 bp element (21), YY1 binding motif (YY1), 5' half of the dyad symmetry (5'), 3' half of the dyad symmetry (3') or octamer motif (oct). Lane 1 contains no recombinant protein.

pSV2neo (data not shown) have no effect on IE promoter activity in undifferentiated T2 cells (Fig. 7). These experiments show that YY1 is able to super-repress the HCMV major IE promoter activity in undifferentiated T2 cells but, as expected, only in the context of a promoter bearing YY1 binding sites. In contrast, whilst YY1 specific repression of the major IE promoter/regulatory region in permissive T2RA cells does consistently occur, this repression is much weaker, suggesting that full repression of the HCMV major IE promoter activity by YY1 is mediated by additional factors specific for undifferentiated T2 cells. This would be consistent with the fact that fibroblast cells, which contain relatively high levels of YY1 protein (data not shown), are permissive for HCMV infection and argues against YY1 acting alone to mediate repression of IE gene expression in non-permissive cells and is also consistent with the belief that YY1 itself does not intrinsically activate or repress promoter activity but facilitates subsequent regulation by other cellular factors (30).

YY1 interacts with factors present in undifferentiated T2 cells

In order to determine if nuclear factors specific for undifferentiated T2 cells (which may act as co-factors for YY1-mediate repression) could interact with YY1 directly, we performed Far-western blotting with nuclear extracts prepared from undifferentiated and differentiated T2 cells using 32 P labelled bacterially produced GST-YY1 fusion protein as a probe. As shown in Fig. 8, proteins which interact directly with YY1 are present in both differentiated and undifferentiated T2 cells. However, two major protein species could be clearly identified

Figure 6. Western blot analysis. 10 μ g nuclear extracts prepared from undifferentiated (lane 1) or differentiated T2 cells (lane 2) were separated by SDS-PAGE, then blotted onto nitrocellulose and probed with a YY1-specific monoclonal antibody (right panel). Mr of markers are shown. The equivalent samples stained with coomassie blue are shown in the left panel to confirm equal protein loading.

which interacted directly with GSTYY1 but not GST alone and which were differentiation-specific as they were not present in RA-induced differentiated T2 cells. This could be consistent with recent observations that ubiquitous cellular factors such as Sp1 and c-myc interact directly with YY1 (31–33). However, as yet, we do not know the identity of the proteins present in the undifferentiated T2 cells which interact with YY1, although our preliminary data suggests that the lower band (in Figure 8 lane 1) is probably YY1 as we have observed homodimerisation of YY1 *in vitro* (data not shown).

Figure 7. Cotransfection assays. The effector plasmid SVYY1 (lanes 2, 4, 6, 8) or control PHK3 alone (lanes 1, 3, 5, 7) were transfected with reporter plasmids pEScat (lanes 1–4) which contains all the putative YY1 binding sites present in the imperfect dyad symmetry and 21 bp repeat elements or pIEP1cat (lanes 5–8), the short deleted IE promoter containing no known YY1 binding sites, in undifferentiated T2 cells (lanes 1, 2, 5 and 6) and differentiated T2RA cells (lanes 3, 4, 7 and 8). Equal amounts of total cell protein were assayed for CAT activity 48 hours post-transfection. Note that samples in differentiated cells (lanes 3, 4, 7 and 8) were diluted 5-fold compared to undifferentiated samples prior to assay. Values are given as relative CAT activities, where a relative CAT activity of 1 is equivalent to 59% CAT conversion and results shown are averages of at least three independent experiments.

DISCUSSION

We have previously shown that the imperfect dyad symmetry in the HCMV modulator region and the 21 bp repeat elements in the HCMV enhancer negatively regulate IE gene expression in undifferentiated non-permissive T2 cells and both sites bind similar if not identical factors (17, 18). We also noticed (17) that the imperfect dyad symmetry and the 21 bp repeat element had considerable homology to the negative regulatory element in the upstream conserved region of the MuLV LTR which has been shown to bind UCRBP (19, 20). Additionally, DNA binding sequence comparison revealed that both the imperfect dyad symmetry and the 21 bp repeat element contain DNA sequences similar to those found in the adeno-associated virus P5 promoter which mediate repression by binding the transcription factor YY1 (23). EMSAs showed that the complexes formed between a YY1 binding motif and nuclear extracts prepared from T2 cells could be specifically competed for, not only by the cold YY1 binding motif as expected, but also by the cold 5' half of imperfect dyad symmetry and the 21 bp repeat motif. These complexes were also similar to those formed by the 5' half of imperfect dyad symmetry or the 21 bp repeat element with T2 nuclear extracts, strongly suggesting that both the 5' half of dyad symmetry and the 21 bp repeat elements of the HCMV major IE promoter/regulator bind YY1. This was confirmed by showing that both sites could bind bacterially expressed YY1 in EMSAs.

YY1 is known to be differentiation specific (19, 26, 34). It decreases in differentiated murine F9 embryonal carcinoma cells induced by RA (19) and is substantially reduced when cultured chicken embryonic myoblasts are allowed to differentiate into myotubes (26). Similarly, we show here that YY1 protein is abundantly present in undifferentiated human T2 cells but decreases upon cell differentiation with retinoic acid. A functional analysis also showed that YY1 was able to super-repress HCMV IE gene expression in undifferentiated non-permissive T2 cells. Interestingly, whilst YY1 does repress the major IE promoter/regulatory region in differentiated T2RA cells, this repression is very much weaker compared to levels of repression by YY1 in undifferentiated T2 cells. This suggests that other,

Figure 8. Far-western blot analysis. 10 μ g of the same nuclear extracts prepared from undifferentiated (lane 1) and differentiated T2 cells (lane 2) shown in figure 6 were separated by SDS-PAGE, then blotted on nitrocellulose and probed with 32 P-labelled GSTYY1 (A) or control GST (B). *Mr* of markers are shown.

so far unidentified, cellular factors specific for undifferentiated T2 cells are necessary to mediate this repression fully. This is consistent with our observations that fibroblast cells contain high levels of YY1 (data not shown) and yet are permissive for HCMV infection and would also explain why the minimal IE promoter, pIEP1cat, is a stronger promoter in fibroblast cells than major IE reporter constructs that contain YY1 binding sites (unpublished observations). It is unlikely that the lower levels of YY1 mediated repression in differentiated T2RA cells is due to lower levels of YY1 expression in these cells as the SV40 early promoter is a much stronger promoter in T2RA cells (unpublished observations). Finally, far-western blot analysis suggested that YY1 does interact with factors which are specifically present in undifferentiated T2 cells, at least *in vitro*, and we believe that these factors may be necessary for the differentiation-specific repression of the IE promoter/regulatory region by YY1.

Our previous analyses of the control of HCMV IE expression have described the presence of a factor/s, MBF1, which also binds to the imperfect dyad symmetry and the 21bp repeat. The presence of this factor/s, an Ets-like factor on the basis of cold competition EMSAs, also correlates with non-permissiveness for IE gene expression (17, 18) and it is likely that, with YY1, this factor/s also plays a role in differentiation-specific repression of IE expression. We still do not know the identity of the MBF1 complex, experiments to define MBF1 and any interaction, both physically and functionally with YY1, are ongoing.

The regulation of viral gene expression by cellular factors YY1 has been shown for other viruses (35–37). YY1 represses the promoter of the human papillomavirus type 18 (HPV-18) and has been suggested as being responsible for repressing transcription of persistent HPV-18 genomes in infected cells in the early stages of tumour development (35). Similarly, YY1 may also play a role in the maintenance of latency of Epstein–Barr virus (EBV), another member of herpesvirus family. In this case, YY1 negatively regulates the promoter of the EBV immediate early protein BZLF1 gene, which mediates the switch from latent to lytic infection (36). Correspondingly, YY1 may also be involved in maintaining the latent state of HCMV in certain cell types. For instance, in monocytic cell lines IE promoter activity is repressed due to similar if not identical factors as seen in T2 cells (38). As monocytes are one site of carriage of HCMV in the peripheral blood but generally IE gene expression is not observed in peripheral blood monocytes of healthy carriers (39), it is possible that YY1 may play a role in maintaining the latency

of HCMV in peripheral blood cells. Negative regulation of viral immediate early and early genes by differentiation specific cellular factors, such as YY1, may be a common mechanism by which viral latency is maintained.

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