PEF-1, an epithelial cell transcription factor which activates the long control region of human papillomavirus type 16, is glycosylated with N-acetylglucosamine

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Infection by human papillomavirus type 16 (HPV-16) has been linked to cervical cancer. The transcription of viral genes in HPV-16 is partially controlled by a number of cellular transcription factors. We have previously identified a novel cellular transcription factor, PEF-1, from its ability to interact with the long control region (LCR) of HPV-16. This factor has a molecular mass of about 110 kDa and binds to a GC-rich sequence in the section of the LCR responsible for cell type-specific transcription from viral DNA. The factor Sp1 has similar properties and also interacts with the HPV-16 LCR. We show that PEF-1 and Sp1 are distinct transcription factors: they recognize different DNA sequences, have different electrophoretic mobilities and different glycosylation patterns. Sp1 is O-glycosylated while PEF-1 appears to have a novel type of glycosylation, as shown by the interaction with pokeweed lectin and by the inhibition of this interaction by tunicamycin.

We have previously identified a cellular transcription factor, PEF-1, which binds to footprint fp5e in the minimal enhancer of the HPV-16 LCR and activates the viral promoter in epithelial cells (Cuthill et al., 1993). The fp5e site (\texttt{ATC-ATCATCGCCCAACGCCTTAACATAC}) contains a PEF-1 site (\texttt{GCGCCAACGCCTTA}) and an overlapping Oct-1 binding site (\texttt{CTATGCC}). PEF-1 and Oct-1 compete for their cognate sites, with PEF-1 up-regulating and Oct-1 down-regulating LCR transcription (Sibbet et al., 1995). PEF-1 has been shown to have a molecular mass of about 110 kDa by UV cross-linking to an oligonucleotide derived from fp5e (Cuthill et al., 1993). Other properties of this protein are not known and, given its epithelium-specific positive role in LCR activation, it is important to further characterize this transcription factor.

A number of transcription factors, including Sp1 (Jackson & Tjian, 1988), SRF (Reason et al., 1992; Schoter et al., 1990), c-Fos, c-Jun and c-Myc (Chou et al., 1995a, b) are glycosylated, as are other nuclear proteins (Kelly & Hart, 1989) including components of the nuclear pore (Sterne-Marr et al., 1992) and RNA polymerase II (Kelly et al., 1993). As glycosylation appears to be a common post-translational modification of transcription factors, we reasoned that PEF-1 may also be glycosylated.

Sp1 is an ubiquitous O-glycosylated transcription factor with a molecular mass of about 110 kDa which binds to GC-rich sites in DNA and interacts with the HPV-16 LCR at...
nucleotides 28–33 (Gloss & Bernard, 1990; Hoppe-Seyler & Butz, 1993, 1994). Sp1 has also been shown to compete with a POU domain factor (Pit-1) for an overlapping binding site in the rat growth hormone gene promoter (Schaufele et al., 1990), similar in this respect to the PEF-1/Oct-1 competition. Sp1 therefore shares some properties with PEF-1, so it is formally possible that PEF-1 could be Sp1 acting at a non-canonical binding site. This possibility was addressed by examining the DNA binding site preferences of PEF-1 and Sp1 and comparing the specific glycosylation of the two proteins.

We report that PEF-1 and Sp1 are clearly separate proteins which have different sequence requirements for DNA binding. The glycosyl groups of PEF-1 are clearly different from that present on Sp1, and inhibition of PEF-1 glycosylation in vivo by tunicamycin indicates that the glycosyl groups of PEF-1 are of a form differing significantly from the previously reported O-linked forms.

Methods

Chemicals. All chemicals were purchased from Sigma–Aldrich, unless otherwise stated, and were of the best grade available.

Cells. HeLa S3 cells were grown in flasks or roller bottles (Falcon) in Special Liquid Medium (Gibco) supplemented with 10% foetal calf serum and 10 mM glutamine. For glycosylation inhibition studies HeLa cells were grown in medium containing tunicamycin. Tunicamycin was dissolved in ethanol to 1 mg/ml and the appropriate amount added to the medium. Control cells were grown with an equivalent amount of ethanol in the medium.

Nuclear protein preparation. Nuclei were prepared by a modification of the method of Hagenbuchle & Wellauer (1992). Briefly, HeLa cells were pelleted and then resuspended in buffer A (60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 15 mM HEPS, 0.8 mM DTT, pH 7.8) containing 0.3 M sucrose and 0.5% Nonidet P40. Approximately 1 × 10^6 cells were collected for each nuclear extract. The cells were then homogenized in a Potter type homogenizer with a motorized Teflon pestle, on ice. The homogenate was layered over a cushion of buffer A with 0.9 M sucrose. This was then centrifuged, the pellet collected, and resuspended in buffer A containing 0.2% Nonidet. The pellet was re-homogenized with three strokes and then re-centrifuged over a sucrose cushion. The resulting pellet was resuspended in 250 µl 0.02 M KCl buffer with the inclusion of a cocktail of protease inhibitors, including 0.5 mM PMSF, and 0.5 µg/ml each of pepstatin, aprotinin, leupeptin and benzamidine; 1 ml 0.6 M KCl was added to this drop-wise with shaking. The extract was incubated on ice for 30 min and then spun for 30 min in a microcentrifuge at 4 °C. The supernatant was collected and used or stored at −80 °C. Extracts prepared in this way contained similar PEF-1 activity to those prepared previously (Sibbet et al., 1995), but had considerably less contaminating protein (results not shown).

Oligonucleotides. All oligonucleotides were synthesized on an Applied Biosystems model 381A DNA synthesizer. Oligonucleotides for electrophoretic mobility shift assays (EMSA) were purified by preparative electrophoresis or COP (Cruachem Ltd, Glasgow) cartridge (as directed by the manufacturer). No difference was noted in the performance of oligonucleotides produced by the two methods. Oligonucleotides were labelled with ^32P using T4 polynucleotide kinase as directed by the manufacturer and annealed by standard methods.

EMSA. This was done as in Sibbet et al. (1995) except that poly(dA-dT) was substituted for poly(dl-dC) as it was found to improve the specificity of binding in this assay (results not shown). EMSAs containing lectins were performed in essentially the same way, except that 10 µg of the appropriate lectin was added in the case of the lectin screening experiments and 0.5–10 µg of the appropriate lectin was added for the comparison of pokeweed and wheatgerm lectins, prior to the initial pre-incubation (15 min on ice) of the nuclear extract with poly(dA-dT) and buffer. For experiments examining the recovery of PEF-1 binding activity from pokeweed lectin inhibition, 5 µg of lectin was added and the appropriate concentration of N-acetylglucosamine was added to the pre-incubation mix. Results were visualized by autoradiography onto film (Fuji X-ray) and by use of a Bio-Rad GS-525 Molecular Image System phosphoimager. Gels were analysed using Molecular Analyst software.

Glycosidase digestion. N-Acetylglucosaminidase (EC 3.2.1.30) digestion of nuclear extract was performed on 10 µl of extract with 8 µl 0.1 M citrate buffer pH 5 and 0.1 U of enzyme and incubated for 1 min to 1 h at room temperature. The activity of the enzyme was followed in a positive control to which p-nitrophenol N-acetylglucosamine, a chromogenic substrate, had been added. The reaction was stopped by adding SDS–PAGE sample buffer and boiling for 5 min. The samples were then run on a 5–15% gradient SDS–PAGE gel and blotted onto PVDF membrane.

SouthWestern blot detection. For SouthWestern detection the proteins were electrobotted by the semi-dry method onto PVDF membrane and the transfer was checked using Ponceau S stain, destained with water and the molecular mass standard tracks were cut off. These were then stained with Coomassie blue. The membrane and the standards were keyed together to allow comparison. Blocking was carried out with 1% BSA and 0.01% Tween 20 in PBS, overnight. The membrane was washed three times with 0.2% BSA–0.01% Tween in PBS; the proteins were denatured on the membrane with 6 M guanidine.HCl (in binding buffer: 25 mM NaCl, 5 mM MgCl_2, 25 mM HEPES, 0.5 mM DTT, pH 7.9) and then renatured by washing progressively with guanidine solutions serially diluted 2-fold, culminating in two washes with binding buffer. The membrane was blocked with the addition of 5 mg/ml dl-dC and then incubated overnight at 4 °C with radiolabelled oligonucleotide under similar conditions used for EMSA (e.g. 5 fmol/ml of labelled oligonucleotide in the binding buffer described above). The membrane was then washed extensively and exposed to a Fuji X-ray film.

Results

Comparison of the DNA binding properties of PEF-1 and Sp1

Both PEF-1 and Sp1 bind to GC-rich sites on DNA. The PEF-1 binding site in the HPV-16 LCR (GGCGCCAC-GGCCCTTA) shows some homology to that of Sp1 (GGG-GGCGGGGC). It was therefore important for us to clearly show that PEF-1 is distinct from Sp1. We constructed oligonucleotides based on the fp5e sequence (fp5e. wt = 5’ atcatctagcggacagcgccttacatc 3’), which contains the PEF-1 binding motif, and with the PEF-1 site mutated to the consensus Sp1 site (fp5e.sp = 5’ atacgcccggccgtcgccttacatc 3’). Oct-1 is unable to bind to either of these oligonucleotides as it requires a longer flanking sequence in the 5’ region and its binding site
PEF-1 is glycosylated

**Fig. 1.** PEF-1 and Sp1 are distinct DNA binding proteins. The contents of the lanes are as follows: HeLa nuclear extract with radiolabelled fp5e.wt (lane 1); HeLa extract pre-incubated with unlabelled fp5e.wt (lane 2); pre-incubated with unlabelled fp5e.sp (lane 3); HeLa nuclear extract with radiolabelled fp5e.sp (lane 4); pre-incubated with unlabelled fp5e.sp (lane 5); pre-incubated with unlabelled fp5e.wt (lane 6). The bottom band detected with the fp5e.sp probe is an uncharacterized complex.

is mutated in fp5e.sp (Sibbet et al., 1995), and therefore it cannot interfere with analysis.

In EMSA the fp5e.wt sequence bound PEF-1 (see Fig. 1, lane 1) and this binding could be competed by a 50-fold excess of unlabelled fp5e.wt oligonucleotide (Fig. 1, lanes 2). The fp5e.sp oligonucleotide bound Sp1 in a complex which migrated more slowly than the fp5e.wt complex (Fig. 1, lane 4); this binding could be self-competed by a 50-fold excess of unlabelled fp5e.sp oligonucleotide (Fig. 1, lane 5).

The binding of fp5e.wt to PEF-1 could be competed out by the addition of unlabelled fp5e.sp (Fig. 1, lane 3), but unlabelled fp5e.wt did not appear to effectively compete with the fp5e.sp oligonucleotide for Sp1 binding (Fig. 1, lane 6). Therefore PEF-1 and Sp1 are different proteins with divergent binding site requirements and although PEF-1 can bind to the Sp1 DNA binding site, Sp1 cannot bind to the PEF-1 sequences.

Given the weak intensity of the PEF-1 complex, PEF-1 appears to be either at a lower concentration than Sp1 or to have a lower affinity for its cognate sequence than Sp1.

**fp5e.wt binding to PEF-1 is inhibited by pokeweed lectin**

Lectins are proteins which specifically bind complex sugars, and can be grouped by their specificity towards particular monomeric sugars (Debray et al., 1981). Lectins can generally substitute for antibodies in a wide range of techniques (for example in EMSA, see below) and the availability of biotinylated lectins allows their use in place of conjugated antibodies, for example in detection on Western blots (Shao & Chin, 1994).

In order to answer the question of whether PEF-1 is glycosylated and, if so, with what type of glycosylation, we pre-incubated HeLa nuclear extracts with a panel of lectins before performing EMSAs. This panel was selected to reflect the major types of known glycosylation; heparin was also included in the panel as it is known to bind to a large range of proteins relatively non-specifically on the basis of the overall charge of the protein.

The strongest inhibition of PEF-1/fp5e binding was found with pokeweed mitogen and heparin (see Fig. 2A, lanes 6 and 9). The heparin inhibition could be reversed by inclusion of a 10-fold greater concentration of non-specific DNA [poly-(dA-dT)] to the reaction (data not shown). This indicates that the effect of the heparin was due to a non-specific interaction with the oligonucleotide rather than interaction with the

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**Fig. 2.** Action of lectins on the PEF-1/fp5e complex. (A) The formation of PEF-1/fp5e.wt complex is strongly inhibited by pre-incubation with pokeweed lectin (lane 6) and heparin (lane 9). Weaker inhibition is also seen with horseshoe crab lectin (lane 14) and elder lectin (lane 15). The contents of the lanes are as follows: fp5e.wt and nuclear extract alone (lane 1); self competition with a 10-fold excess of unlabelled fp5e.wt (lane 2); pre-incubated with soybean lectin (lane 3); kidney bean lectin (lane 4); wheatgerm lectin (lane 5); pokeweed lectin (lane 6); pea lectin (lane 7); lentil lectin (lane 8); heparin (lane 9); Con A (lane 10); lanes 11, 12 and 13 as lanes 1, 2 and 6; horseshoe crab lectin (lane 14); elder lectin (lane 15). Ten µg of lectin was used in each case. (B) Recovery of PEF-1 binding by addition of N-acetylglucosamine to PEF-1 inhibited by 5 µg pokeweed lectin. HeLa nuclear extract and labelled fp5e.wt oligonucleotide (lane 1), self competition (lane 2). HeLa extract incubated with 5 µg pokeweed lectin (lane 3). HeLa extract pre-incubated with 5 µg pokeweed lectin and N-acetylglucosamine at 10 µM (lane 4), 60 µM (lane 5), 0.1 mM (lane 6), 0.6 mM (lane 7) and 1 mM (lane 8). Densitometric analysis was carried out on the exposed film using a PDI 420oe scanner and the bands were auto-identified and quantified using PDI Quality One (version 2.7).
Strong inhibition by pokeweed is suggestive of the presence on PEF-1 of N-acetylglucosamine (Katogirir et al., 1983) in the form of chitobiose (Yamaguchi et al., 1996). Poor inhibition by wheatgerm lectin (Fig. 2A, lane 5) indicates that PEF-1 glycosylation is unlikely to be the same as that previously found on Sp1 (Jackson & Tjian, 1989). No inhibition of PEF-1/fp5e.wt complex formation was seen with soyabean, kidney bean, wheatgerm, pea, lentil and Con A lectins (Fig. 2A, lanes 3, 4, 5, 7, 8 and 10 respectively), showing that PEF-1 does not contain sugar groups recognized by these lectins.

However, inhibition was seen with horseshoe crab lectin and particularly with elder lectin (Fig. 2A, lanes 14 and 15). These lectins have affinities for sialic acid residues, and thus these results suggest that the PEF-1 glycosylation contains sialic acid. These sugar groups are often found in the terminal positions of complex glycosyl structures, suggesting that the glycosylation is not a simple single O-linked N-acetylglucosamine.

Recovery of PEF-1 binding by the addition of N-acetylglucosamine to pokeweed-inhibited PEF-1

In order to demonstrate the specificity of pokeweed lectin inhibition of PEF-1 DNA binding, competition EMSAs were carried out with increasing concentrations of N-acetylglucosamine, the simple sugar which binds to pokeweed lectin. With increasing concentrations of N-acetylglucosamine, PEF-1/fp5e.wt binding activity could be recovered in a dose-dependent manner (Fig. 2B). This shows that N-acetylglucosamine and the glycosylation moiety of PEF-1 can compete for the pokeweed lectin binding site and that therefore the inhibition of PEF-1 binding is not due to a non-specific interaction between the lectin and PEF-1 protein. A non-specific interaction with the DNA probe is ruled out by the inability of excess non-specific oligonucleotide [oligo(dA·dG) or oligo(dA·dT)] to interfere with pokeweed lectin inhibition (data not shown).

The glycosylation of PEF-1 differs from that of Sp1

Sp1 is glycosylated by O-linked N-acetylglucosamine (Jackson & Tjian, 1988) and, as expected, wheatgerm lectin inhibited its binding to its cognate DNA site (Fig. 3B). We investigated the inhibition kinetics of pokeweed and wheatgerm lectins for PEF-1 and Sp1 DNA binding. PEF-1 binding to fp5e.wt was inhibited to 40% by 1 µg of pokeweed lectin and completely abolished by 2 µg (Fig. 3A). Wheatgerm lectin had no detectable effect on PEF-1/fp5e.wt complex even at 10 µg (Fig. 3A). The reverse was true for Sp1. Sp1 binding to fp5e.sp was inhibited to 60% by 1 µg of wheatgerm lectin and almost completely abolished by 2 µg, while pokeweed lectin had no effect (Fig. 3B).

These results show that the glycosylation groups on PEF-1 and Sp1 are distinct, and confirm that PEF-1 and Sp1 are different entities.

In vivo inhibition of PEF-1 N-linked glycosylation by tunicamycin in HeLa nuclear extracts

Tunicamycin inhibits the first step in N-linked glycosylation of proteins (Heifetz et al., 1979), and N-linked protein glycosylation can be completely inhibited in vivo by culturing cells in the presence of tunicamycin.

Although treatment with tunicamycin has been shown to have secondary effects such as apoptosis (Driu et al., 1997; Perez-Sala & Mollinedo, 1995), we established conditions which did not impair cell viability. HeLa cells were cultured with increasing concentrations of tunicamycin in the medium to establish the concentration which could be tolerated by the cells. No cell death was seen up to 5 µg/ml, consistent with the activity of tunicamycin in other cell types (Tiganis et al., 1992; Larsson et al., 1993) (data not shown). A final concentration of 6·25 µg/ml for 24 h was chosen as at this concentration tunicamycin inhibited glycosylation, as measured by reduced binding of biotinylated pokeweed lectin to cellular proteins on Western blots (data not shown), but did not produce extensive cell death. The cells were then mechanically harvested and nuclear extracts prepared. As expected pokeweed lectin noticeably reduced PEF-1 binding to fp5e.wt in a non-treated
PEF-1 is glycosylated

Fig. 4. PEF-1 and Sp1 complex formation in nuclear extracts from tunicamycin-treated and control cells. (A) Nuclear extracts from tunicamycin-treated and control HeLa cells were incubated with fp5e.wt, with or without unlabelled competitor (self), and with or without pre-incubation with pokeweed lectin. Densitometric analysis was carried out on the exposed film using a PDI 420oe scanner and the bands were auto-identified and quantified using PDI Quality One (version 2.7). (B) Nuclear extracts as above were incubated with fp5e.sp. The bottom band detected with the fp5e.sp probe is an uncharacterized complex.

nuclear extract (Fig. 4A, control lanes). On the contrary, little or no inhibition by pokeweed lectin was observed in a tunicamycin-treated extract (Fig. 4A, tunicamycin lanes), indicating that PEF glycosylation had been abolished by the drug, and suggesting that the glycosyl groups of PEF-1 may be in a N-linked form. The overall DNA binding of PEF-1 in the tunicamycin-treated extracts was reduced by approximately 3-fold. This reduction is likely to be due to the general inhibition of protein synthesis by tunicamycin, as Sp1 binding was also reduced by approximately 2-fold (Fig. 4B), although it is not expected to be affected specifically by tunicamycin. It has to be noted that tunicamycin treatment did not affect the ability of PEF-1 to bind fp5e.wt. The mobility of the complex was, however, slightly increased, possibly reflecting a decrease in the glycosylation of the protein (Fig. 4A). The mobility of Sp1 was unchanged, confirming that tunicamycin did not specifically affect Sp1.

In vitro removal of terminal N-acetylglucosamine groups

Terminal N-acetylglucosamine groups can be removed from glycoproteins by digestion with N-acetylglucosaminidase. We digested protein extracts with N-acetylglucosaminidase and monitored the activity of the enzyme with p-nitrophenol-substituted N-acetylglucosamine, a small chromogenic substrate. Digested and control protein extracts were Western blotted and the presence of PEF-1 was detected by its binding to radiolabelled fp5e.wt oligonucleotide.

In the extracts treated with N-acetylglucosaminidase PEF-1, detected by the binding of fp5e.wt oligonucleotide, had a faster electrophoretic mobility, suggesting that a significant part of the protein’s glycosylation has been removed by this enzyme (Fig. 5).

The treatment of protein extracts with N-acetylglucosaminidase (room temperature and low pH for up to 1 h) precludes subsequent analysis by EMSA.

Discussion

PEF-1 is a cellular protein that binds to the LCR of HPV-16 and activates the viral promoter in epithelial cells (Cuthill et al., 1993; Sibbet et al., 1995). PEF-1 and the ubiquitous transcription factor Sp1, which also binds the HPV-16 LCR (Hoppe-Seyler & Butz, 1994), share a number of features: their molecular mass is approximately 110 kDa and both bind to GC rich DNA sites. Here we further characterize PEF-1 and demonstrate that PEF-1 is glycosylated by a N-acetylglucosamine structure which differs from that of Sp1. This glycosylation pattern and the specific DNA binding characteristics of PEF differentiate unambiguously PEF-1 from Sp1.

DNA binding characteristics of PEF-1

PEF-1 and Sp1 clearly have different requirements for DNA binding, highlighted by their respective complex formation and competition with fp5e.wt and fp5e.sp. The weak intensity of the PEF-1 complex suggests that PEF-1 is present at low concentrations in the nucleus or has a lower affinity for its binding site in fp5e.wt. It is possible that the fp5e site in the
HPV-16 LCR is not the preferred binding sequence of PEF-1 and that PEF-1 binds more avidly to sites in cellular genes. While Sp1 does not recognize the PEF-1 site, PEF-1 binds to both its own site and to the Sp1 site in Fp5e.sp. Thus although the sequences surrounding the Sp1 binding site may influence the interaction with PEF-1, PEF-1 may also interact with Sp1 binding sites in cellular genes and compete with Sp1 itself.

**Glycosylation pattern of PEF-1**

One of the classical methods for studying sugar groups on proteins is through the use of lectins. Lectins are a group of proteins, found mainly in plants, which bind specifically to different sugars, and thus they can be used to discriminate between different patterns of protein glycosylation (Cummings et al., 1989). We have exploited the discriminatory power of lectins to show that PEF-1 is glycosylated with N-acetylglucosamine in a previously unreported pattern for a transcription factor, and differs from that of Sp1 which exhibits the more typical O-linked glycosylation.

This conclusion is supported by several observations.

1. **Pokeweed lectin**, which binds specifically to N-acetylglucosamine (Yamaguchi et al., 1996), inhibits PEF-1 complex formation. Digestion with N-acetylglucosaminidase changes the electrophoretic mobility of PEF-1.

2. **N-Acetylglucosamine**, the prototype ligand of pokeweed lectin, restores PEF-1 complex formation in the presence of the lectin; N-acetylglucosamine clearly competes specifically with the glycosylation moiety of PEF-1 for binding to pokeweed lectin, and thus confirms PEF-1 glycosylation by a N-acetylglucosamine group.

3. Sp1 complex formation is inhibited by wheatgerm lectin but not by pokeweed lectin in accordance with the previously reported O-linked glycosylation pattern of Sp1 (Jackson & Tjian, 1988). Like pokeweed lectin, wheatgerm lectin binds to N-acetylglucosamine (Shao & Chin, 1994), but primarily in its O-linked form (Chou et al., 1995a; Kelly & Hart, 1989; Sterne-Marr et al., 1992). Although some cross-reactivity with N-linked sugars has been demonstrated (Kawashima et al., 1990), the affinity of wheatgerm lectin for N-linked glycosylation is much lower than for O-linked groups (Yamamoto et al., 1981). The kinetics of inhibition of PEF-1 and Sp1 complexes by the two lectins clearly show that the glycosylation patterns of the two proteins are different, and that PEF-1 may be glycosylated in a way which fundamentally differs from the canonical O-linked structures previously described.

4. **Tunicamycin** inhibits the addition of asparagine-linked N-acetylglucosamine to protein and this inhibitor is routinely used as a probe for the presence of N-linked glycosyl structures on glycoproteins (Cummings et al., 1989). The formation of the PEF-1-Fp5e complex in tunicamycin-treated nuclear extract is no longer prevented by pokeweed lectin. Thus an asparagine N-linked N-acetylglucosamine group would appear to have been removed from PEF-1 and pokeweed lectin can no longer interact with the protein. However, PEF-1 can still bind to its DNA site, which demonstrates that PEF glycosylation is not essential for DNA binding activity.

5. The binding of horseshoe crab lectin and particularly elder lectin (Codongo et al., 1992; Emig et al., 1995; Do Valle Matta et al., 1995) indicates the presence of sialic acid residues on PEF-1; these groups are commonly found in the terminal positions and have been previously identified in the N-linked glycosyl structures in the nuclei of HeLa cells and of rat brain cells (Codongo et al., 1992). This result indicates that the glycosylation of PEF-1 is not the same as the simple O-linked single N-acetylglucosamine moiety which has been reported for some other nuclear glycoproteins (Hart et al., 1989).

While it is difficult to interpret the data from lectin binding experiments in terms of a detailed carbohydrate structure, as many of the lectin interactions with complex sugar chains have not been adequately elucidated, our results strongly suggest that PEF-1 is glycosylated in a form not previously identified on transcription factors and the glycosylation is likely to be of a complex type, containing N-acetylglucosamine repeats as described for HeLa nuclear glycoproteins.

**Functional significance of PEF-1 glycosylation**

The functional significance of PEF-1 glycosylation is not known. Although non-glycosylated PEF-1 can still bind its target site in the HPV-16 LCR, it remains to be established whether this interaction can still lead to transcription activation of the LCR. In the case of Sp1, it has been reported that the glycosylated form of the protein has a greater ability to transactivate than the non-glycosylated form (Jackson & Tjian, 1988).

The glycosylation state of a glycoprotein may not only affect protein functions but also feed-back to control the expression of that protein. This is the case for the O-linked glycosylation of p53 in the epitope recognized by monoclonal antibody PAB421. Thus, the DNA binding activity of p53 is enhanced when the PAB421 epitope is masked by glycosylation, and it appears that glycosylation at this site may be responsible for long-term activation and resistance to degradation (Shaw et al., 1996). Likewise reduction of glycosylation on Sp1 is also associated with decreased transcriptional activity (Jackson & Tjian, 1988) and increased degradation by proteasomes (Han & Kudlow, 1997).

The c-Myc protein is also O-glycosylated in its transactivation domain (Chou et al., 1995a) on threonine 58, a known phosphorylation site and a hot spot for c-Myc mutation (Chou et al., 1995b; Takahiro et al., 1993), suggesting a functional significance for this modification.

In addition to the possible control of transcription factors, glycosylation appears to be involved also in the control of viral DNA replication. Adenovirus type 5 and simian virus 40 DNA replication is reduced by inhibition of N-linked glycosylation with tunicamycin (Haung & Hsu, 1991).

The above observations raise the possibility that transcription and replication factors may have multiple layers of
control, with short-term control being mediated by phosphorylation and long-term control by glycosylation. In fact there appears, so far, to be a reciprocal relationship between phosphorylation and glycosylation (Chou et al., 1995b).

The importance these controls have for small DNA viruses is obvious as the virus is forced to co-opt the cellular replication and transcription machinery for its own use. In particular, HPV is crucially dependent on epithelial cell transcriptional factors for expression of its own genes, and PEF-1 appears to be one of the major players in the epithelium-specific control of HPV-16 gene expression. Although the role of post-translational glycosylation in PEF-1 transcriptional activity remains to be elucidated, the possibility that it provides a novel way of fine tuning is attractive and deserves further investigation.

We would like to thank Professor J. Wyke and Dr Iain Morgan for their helpful comments and Dr G. Sibbet for help with techniques. Thanks are due to the Medical Research Council for supporting this work.

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Received 1 May 1998; Accepted 10 July 1998