Virology 394 (2009) 194–199

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

The human papillomavirus 16 E2 protein is stabilised in S phase

Cecilia Johansson [a], Sheila V. Graham [b], Edward S. Dornan [a], Iain M. Morgan [a,*]

[a] Institute of Comparative Medicine, University of Glasgow Faculty of Veterinary Medicine, Glasgow, G61 1QH, UK
[b] Division of Infection and Immunity, University of Glasgow Faculty of Biomedical and Life Sciences, Glasgow, G12 8TT, UK

ARTICLE INFO

Article history:
Received 6 April 2009
Returned to author for revision 17 June 2009
Accepted 26 August 2009
Available online 24 September 2009

Keywords:
Papillomavirus
E2
Replication
Stability
Phosphorylation
Cell cycle

ABSTRACT

The human papillomavirus 16 E2 protein regulates transcription from, and replication of, the viral genome and is also required for segregation of the viral genome via interaction with mitotic bodies. To regulate DNA replication E2 interacts with sequences around the origin of replication and recruits the viral helicase E1 via a protein-protein interaction, which then initiates viral genome replication. The replication role of E2 must originally function in a host cell S phase. In this report, we demonstrate that E2 is stabilised in the S phase of the cell cycle and that this stabilisation is accompanied by an increase in phosphorylation of the protein. This increased phosphorylation and stability are likely required for optimum viral DNA replication and therefore identification of the enzymes involved in regulating these properties of E2 will provide targets for therapeutic intervention in the viral life cycle. Preliminary studies have identified E2 as a Cdk2 substrate demonstrating this enzyme as a candidate kinase for mediating the *in vivo* phosphorylation of HPV16 E2.

Introduction

The papillomavirus E2 protein has a modular structure; a carboxyl terminal dimerisation and DNA binding domain, a middle hinge region and an amino terminal transcriptional activation/E1 interacting domain (Steger et al., 1996). HPV E2 dimerises and binds to four 12 bp palindromic target sequences within the long control region (LCR) of the virus; the organisation of these target sequences is conserved amongst all HPV. Following interaction with the LCR, the E2 protein can regulate transcription from the adjacent E6 promoter (Steger and Corbach, 1997). Most reports describe repression of HPV transcription by E2, but studies in primary cells demonstrate that low E2 levels activate transcription while increased levels result in transcriptional repression (Bouvard et al., 1994). Three of the E2 target sequences in the LCR surround the viral origin of DNA replication (ori). Following binding to their target sites at the ori, a physical interaction between the amino terminal domain of E2 and the viral DNA replication factor E1 results in recruitment of E1 to the origin of replication (Benson and Howley, 1995; Mohr et al., 1990). E1 then interacts with the AT rich ori and forms a di-hexameric complex with helicase activity that unwinds the double stranded DNA viral genome and recruits factors to initiate DNA replication (Hu et al., 2006; Loo and Melendy, 2004; Masterson et al., 1998; Schuck and Stenlund, 2005). Another crucial role for E2 in the viral life cycle is to mediate viral genome segregation during cell division. The E2 protein is loaded onto mitotic chromatin by the cellular protein ChlR1 (Parish et al., 2006) and interaction with the cellular factor Brd4, which is associated with mitotic chromatin, regulates viral genome partition for some E2 proteins; the amino terminal domain of E2 interacts with the mitotic chromatin via Brd4 while the carboxyl terminal domain interacts with the viral genome (You et al., 2004). Brd4 is not required for the genome segregation function of all E2 proteins (McPhillips et al., 2006) and E2 can interact with alternative mitotic bodies and proteins including tubulins and TopBP1 (Boner et al., 2002; Donaldson et al., 2007; Van Tine et al., 2004).

All of these functions of E2 are required for a productive viral life cycle and therefore their disruption would be anti-viral. One way to regulate the E2 protein and disrupt its function is to target the stability of the protein itself. For BPV1 E2, protein stability is mediated via phosphorylation in the hinge region of the protein (Penrose and McBride, 2000), while for HPV18 E2, a sequence in the amino terminal domain of the protein is essential for regulating protein turnover (Bellanger et al., 2001). Previous work from our lab has established that the HPV16 E2 protein half life is 45 min, which is reduced following UVB irradiation although the phosphorylation status of this protein has not been investigated (Taylor et al., 2003). In this report, we have investigated the protein turnover of HPV16 E2 during the cell cycle with particular emphasis on the S phase. Previously, we have shown that U2OS cells can stably express the HPV16 E2 protein (from now on denoted E2) (Taylor et al., 2003) and also support E1-E2 mediated DNA replication (Boner et al., 2002). This represents the only known system in which the cell cycle control of HPV16 E2 expression can be studied. The U2OS model system, that supports E2 mediated DNA replication, was therefore used to investigate the cell cycle control of E2 protein levels. There is a peak of E2 protein in S phase mediated by increased protein stability. E2 is phosphorylated throughout the cell cycle and results suggest that phosphorylation is

* Corresponding author.
E-mail address: i.morgan@vet.gla.ac.uk (I.M. Morgan).

0042-6822/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2009.08.046

increased during S phase. This increased level and modification of E2 in S phase likely contribute to the replication function of the protein, therefore identification of the enzymes responsible will reveal antiviral therapeutic targets. Preliminary results suggest that Cdk2 may be mediating this phosphorylation.

Results and discussion

E2 protein levels peak in S phase

All experiments were performed using the previously described U2OS B3 clone that expresses HPV16 E2 (Taylor et al., 2003), although some were repeated in other clones with similar E2 levels, giving identical results. Throughout this report, these will be referred to as U2OS-E2 and control cells U2OS-Vec, the latter were prepared at the same time as the E2 line with a vector not encoding E2. In order to synchronise the cells, they were treated with the DNA polymerase inhibitor aphidicolin (5 μg/ml) for 16 h. This treatment arrests the cells at the G1-S phase transition of the cell cycle (called G1 from now on). The drug was then removed, allowing for cell cycle progression, and cells were harvested at different time points and protein extracts prepared. E2 protein levels were investigated using western blotting. Cells arrested in G1 showed reduced levels of the E2 protein compared to cycling cells (Fig. 1A). Following release from aphidicolin, E2 levels were increased at 4 h, after which they were reduced by 8 h before returning to basal levels by 12 h. This experiment was repeated several times with very similar results. The blots were quantitated and the results are shown in Fig. 1B where it is evident that there is an increase of the E2 protein levels 4 h following aphidicolin release. To confirm that the cells at 4 and 8 h time points represented S phase populations, flow cytometry was carried out and the results shown in Fig. 1C; this experiment was repeated another two times with essentially the same results. Substantial numbers of the cells are in S phase at the 4 and 8 h time points and the E2-expressing cells seem to progress into and through S phase at a slightly slower rate than control cells. However, this slight lack of cell cycle co-ordination could not explain the differences in E2 protein levels observed in Fig. 1A and cell growth studies demonstrate that E2-expressing cells grow at the same rate as control cells (not shown). It should be noted that at the 4 h time point the S phase is not so pronounced, but as there is a G2 peak it demonstrates the cells are progressing through the cell cycle and although the G1 peak is large, it is likely that a lot of these cells have entered S phase.

To confirm that the E2 protein increase was a post-transcriptional event, RNA levels were measured throughout the cell cycle (Fig. 2A). Although there is a small increase in E2 RNA levels at 4 h, it was not reflective of the increase in protein levels, nor was it sustained to the 8 h time point. Immunofluorescence studies also demonstrated an increased E2 protein level during S phase (Fig. 2B). This technique detects the total amount of the E2 protein in the cell, ruling out differences in solubility of the E2 protein that could vary over the cell cycle. While not quantitative, it is clear that there is a peak in the E2 signal detected by immunofluoresence at the 4 h time point. Taken together, these results demonstrate that the E2 protein levels are increased in the S phase of the cell cycle, and that this increase is regulated at a translational or post-translational level.

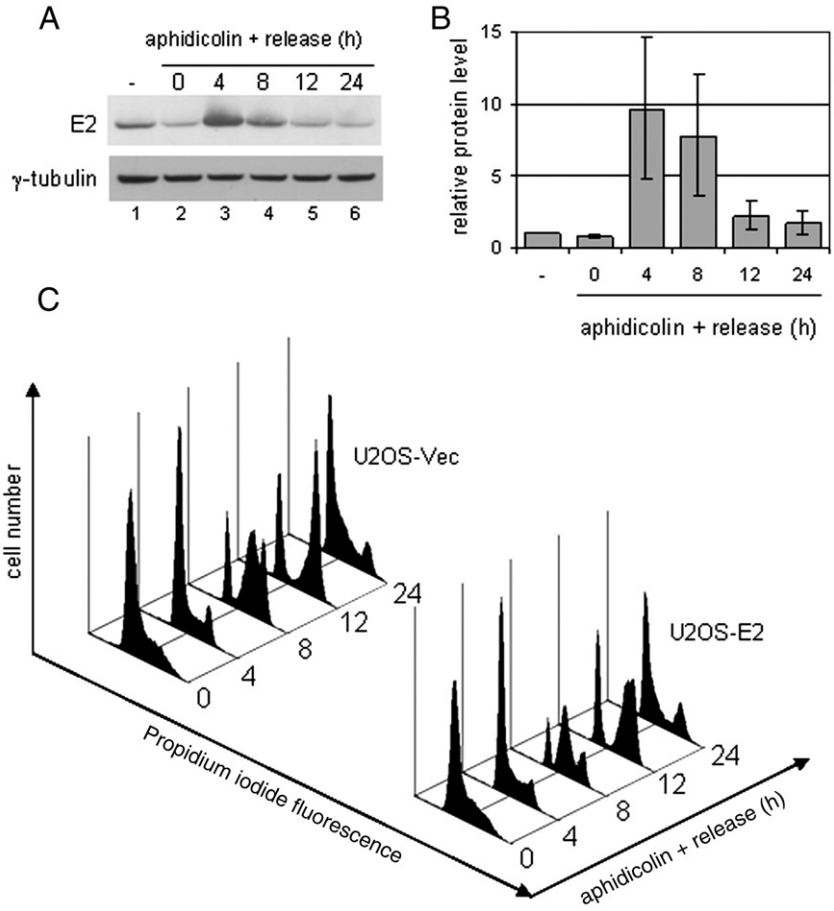

Fig. 1. E2 protein levels peak in S phase. (A) E2-expressing cells were treated with 5 μg/ml aphidicolin for 16 h, washed and harvested at time points indicated. "-" represents non-treated cells. E2 and γ-tubulin were detected with western blot using the TVG261 and GTU88 monoclonal antibodies, respectively. (B) Blots in A were quantified and E2 levels are shown normalised to γ-tubulin. (C) Progression of the cell cycle in E2-expressing and non-expressing cells was verified by propidium iodide staining and flow cytometry.

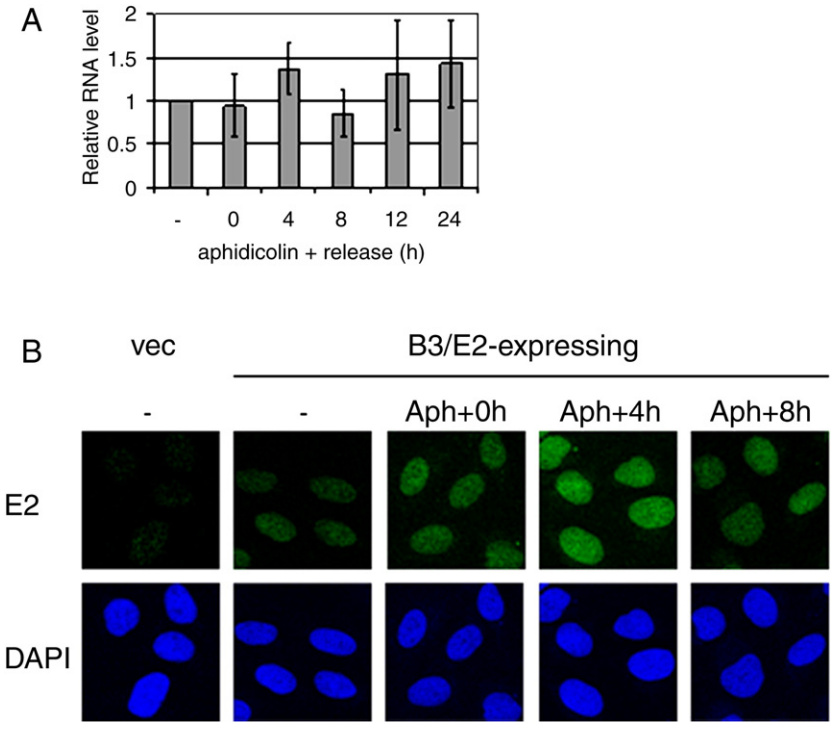

Fig. 2. Increase in S phase E2 is not due to RNA levels or E2 solubility. (A) E2 RNA levels were detected using real-time PCR (probe and primer sets are available upon request). The graph represents 3 experiments with results normalised to β-actin. (B) Cells grown on coverslips were fixed and stained with the TVG261 monoclonal E2 antibody and anti-mouse-FITC conjugated antibody. Vec represents control cells stably transfected with an empty vector not expressing E2.

E2 is stabilised in S phase and has a higher affinity for chromatin

To investigate whether the increased E2 protein level is due to increased stability, E2-expressing cells were synchronised in G1 and S phase (0 and 4 h time point respectively) and thereafter treated with the protein synthesis inhibitor cycloheximide for indicated time periods. It is clear that there is an increased E2 stability in the S phase sample (Fig. 3A, lower panel). The experiment shown is an example of at least three independent experiments and the quantitated results, with associated standard errors, are shown in Fig. 3B. In G1 cells, the half life of E2 was 45 min, which was similar to that of a non-synchronised population (Taylor et al., 2003, and data not shown).

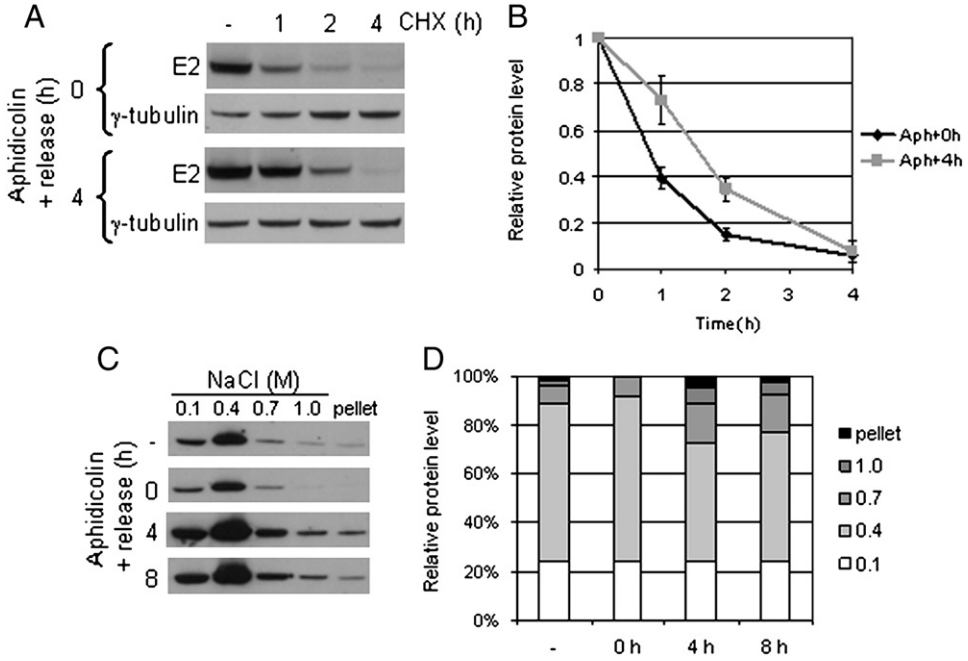

Fig. 3. The E2 protein is more stable in S phase and has increased affinity for chromatin. (A) E2-expressing cells were synchronised with aphidicolin as in Fig. 1 and treated with 10 μg/ml cycloheximide for indicated time periods. E2 and γ-tubulin were detected using western blot. "0" represents aphidicolin arrested cells, "4" represents cells 4 h following release. (B) Blots were quantified and E2 levels relative to pre-cycloheximide treatment were plotted. The graph represents the standard error of the mean of three independent experiments. (C) Proteins from synchronised E2-expressing cells were serially extracted using NP40 lysis buffer with salt concentrations ranging from 0.1 to 1 M as described (Donaldson et al., 2007) and E2 detected using western blot. "0" represents aphidicolin arrested cells, "4" and "8" represent cells 4 and 8 h following release; "–" represents non-treated cells. (D) Blots in A were quantified and E2 levels in each salt fraction were plotted as a percentage of the E2 total for each time point.

When cells entered S phase, the half life of E2 was extended to approximately 90 min. This indicates that the increase in E2 protein levels during S phase was mediated by increased protein stability.

Previous studies have shown that E2 with a higher affinity for chromatin is more stable (Donaldson et al., 2007). It was therefore possible that the enhanced E2 levels detected in S phase were due to elevated levels of soluble E2 extracted from the chromatin. To test this, E2 was serially extracted from the nuclear pellets of synchronised cells using increasing concentration of salt (Fig. 3C). In the S phase samples (4 and 8 h), E2 showed increased affinity for chromatin, indicated by the increased amount of extracted protein in the high salt fractions. Blots were quantified and graphically represented in Fig. 3D where the increase in the percentage of E2 in the higher salt fractions at the later time points was evident. Looking cumulatively at the level of E2 protein in each of the salt extracts and the cell pellet, it is again clear that the total level of E2 protein is elevated in the S phase extracts. This is in agreement with the results shown in Figs. 1 and 2 where increased E2 levels in westerns and immunofluorescence, respectively, are detected.

The levels of E2 phosphorylated protein increase in S phase

In Fig. 1A at the 4 and 8 h time points, there is evidence for a sub-population of E2 protein with slower mobility that could represent modified versions of the protein. BPV1 E2 has two major phosphorylation sites that regulate turnover of the protein; targeting of residue 301 by casein kinase II results in a conformational change resulting in protein degradation (Penrose et al., 2004). These residues are not conserved in the high risk HPV E2 proteins nor is the mechanism of protein turnover. The amino terminal domain of HPV18 E2 is required for proteasome mediated degradation (Bellanger et al., 2001), although the signals that trigger this degradation remain unknown. There have been no reports to date investigating the phosphorylation status of the HPV16 E2 protein throughout the cell cycle and how this might relate to the control of protein turnover. As phosphorylation clearly regulates the turnover of other E2 proteins, the phosphorylation status of E2 in untreated, G1 and S phase cells was investigated using the Qiagen Phosphoprotein Purification Kit. Protein extracts from E2-expressing cells were prepared from untreated and aphidicolin arrested cells as well as cells 4 h following release from aphidicolin using a lysis buffer provided in the kit. These lysates were then processed and analysed as described in Fig. 4A. Input levels of E2 were determined prior to loading onto the phospho-protein purification column using western blotting as shown in Fig. 4B. With the Qiagen buffer, the increase in E2 levels in S phase is not as obvious as in Fig. 1 and therefore identical cell samples from the same experiment were prepared using NP40 buffer (used in the experiments shown in Fig. 1) and blotted for E2 levels. This is shown in Fig. 4B where it is clear that there is the previously observed peak in S phase levels of the E2 protein γ-tubulin controls to confirm equal loading. The protein lysates prepared using the Qiagen buffer were then added to phosphor-affinity columns and fractions collected as flow through (F), or following elution (E). Phosphorylated E2 could be detected in G1 and S phase eluates, and also in untreated (−) cells (Fig. 4B). There was more E2 in the eluate of the S phase sample relative to the input sample suggesting increased phosphorylation of E2 in S phase. This increase was robust and reproducible in several experiments, and therefore, densitometry was carried out on three independent experiments and the levels of E2 in the eluate are shown graphically in Fig. 4C with standard error bars. TCA precipitation was used to reduce the volume of the flowthrough protein sample before western blotting and this resulted in variable detection of E2 due to difficulties in reproducible precipitation of the protein samples. To confirm that the phosphor-protein columns were operating successfully, a known phosphorylated cellular protein, SF2/ASF (Hanamura et al., 1998) was included as a control and the results are shown in Fig. 4D for one of the experiments summarised in Fig. 4C (this was done with all experiments and identical results obtained). Clearly, the SF2/ASF is retained on the phosphor-column as it is detected in the eluted fraction. This confirms the ability of the columns to bind known phosphor-proteins. The increased E2 phosphorylation could explain the enhancement of E2 protein stability as modification could either directly regulate E2 turnover or be responsible for the association of E2 with additional cellular complexes in which the protein is more stable.

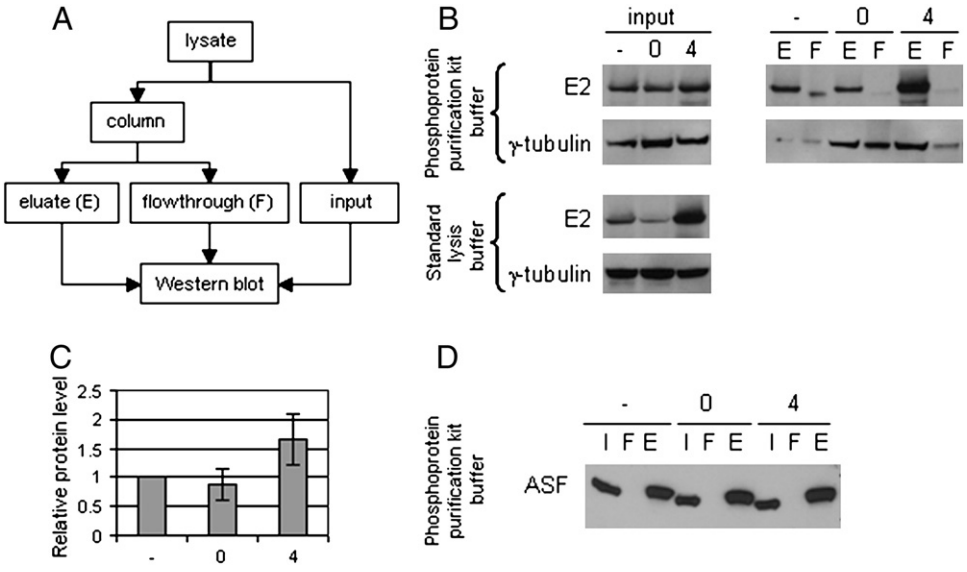

Fig. 4. E2 protein is phosphorylated *in vivo*. (A) Schematic diagram showing phosphoprotein purification using the Phosphoprotein Purification Kit (Qiagen) according to the manufacturer's instructions. (B) The lysates were loaded onto columns, eluted, TCA precipitated and western blotted for E2. "0" represents aphidicolin arrested cells, "4" represents cells 4 h following release, "–" represents non-treated cells. "E" represents the eluate from the phosphoprotein affinity column; "F" represents the flow through. Input lysates from the kit as well as samples lysed in our standard buffer (Figs. 1–3) are shown for comparison (both extracts prepared from the same treated cell population). (C) Bands were quantified and the amount of E2 in the eluted fractions relative to untreated cells (−) was plotted. The graph represents the mean and standard error of the mean from three experiments. (D) Input (I), eluate (E) and flowthrough (F) of one of the experiments summarised in panel C were probed with an antibody against the known phosphor-protein SF2/ASF to confirm that phosphorylated proteins were sticking to the columns.

Elevated levels of Cdk2 activity in early S phase promote loading of proteins such as Cdc45 onto origins to initiate DNA replication and phosphorylation of the viral E1 protein by Cdk2 is required for the activation of HPV DNA replication (Ma et al., 1999). It was therefore possible that Cdk2 is involved in the phosphorylation of the E2 protein during S phase as E2 has a Cdk consensus sequence (TP) in the DNA binding domain at amino acids 286–287 (Fig. 5A). This was investigated *in vitro* using GST fusion proteins (Fig. 5B) and purified recombinant Cdk2. Fig. 5C demonstrates that E2 was a substrate of Cdk2 *in vitro* and that mutation of T286 to alanine resulted in a significant reduction in E2 phosphorylation. However, a residual level of phosphorylation remained suggesting an additional non-consensus Cdk2 target within E2 and this was supported by an amino terminal deletion mutant lacking S286 that was still phosphorylated. This indicates that there are at least two Cdk2 target sequences in E2. A T286A mutant of E2 expressed *in vivo* had no alteration in S phase accumulation or protein stability indicating that this residue by itself is not responsible for regulating E2 stability.

The results presented in this report demonstrate that HPV16 E2 is stabilised in the S phase of the cell cycle, that there is increased phosphorylation of the protein in S phase, and that it is a Cdk2 substrate, at least *in vitro*. Clearly, all of these aspects may combine to explain our observations; increased phosphorylation of E2 may stabilise the E2 protein in S phase. Phosphorylation of the HPV E1 protein regulates the nuclear shuttling of this protein (Yu et al., 2007) although with E2 this does not seem to be the case as it is not detected in the cytoplasm at any point in the cell cycle (Fig. 2B). Upon entry into S phase, several proteins involved in the initiation of DNA replication are phosphorylated by kinases such as Cdk2 and these modifications are essential for the replication function (Aleem et al., 2004). Elevated levels of Cdk2 activity in early S phase promote loading of proteins such as Cdc45 onto origins to initiate DNA replication and phosphorylation of the viral E1 protein by Cdk2 is required for the activation of HPV DNA replication (Ma et al., 1999). We have identified E2 as a Cdk2 substrate *in vitro* and mutation of a Cdk2 target sequence resulted in reduction of Cdk2 phosphorylation, but *in vivo*, mutation of the target residue (T286A) did not alter E2 stability (not shown).

It is also possible that other modifications of E2 play a role in regulating protein stability and function throughout the cell cycle. For example, recent studies demonstrate that cellular sumoylation levels can influence E2 stability (Wu et al., 2009) and this could alter throughout the cell cycle. Alternatively, additional phosphorylation events may contribute to the regulation of E2 stability. Future work will focus on identifying the modified residues on E2 and how they regulate stability. This will allow the identification of the enzyme responsible for modification and this will represent a target for regulating E2 protein levels and therefore target HPV infections.

Materials and methods

Cell culture

E2-expressing U2OS cells (clone B3) and cells containing the empty vector (Taylor et al., 2003) were grown in DMEM+GlutaMAX containing 10% FCS. For G1 arrest, cells were treated with 5 μg/ml Aphidicolin for at least 16 h. Cells were then washed to allow progression of the cell cycle and harvested at indicated time points. To determine protein half life, cells were treated with 10 μg/ml of cycloheximide for indicated time periods.

Flow cytometry

Cells were harvested by trypsinisation followed by 2 washes with PBS. Cell pellets were resuspended in 500 μl of PBS, placed on ice and fixed with 4.5 ml of ice cold 70% ethanol added drop wise over a vortex at low speed. The cells were incubated on ice for 30 min, or if not required immediately, stored at − 20 °C. For DNA analysis, the cells were stained with Propidium Iodide. The cells were washed twice with PBS and resuspended in 500 μl of PBS with 2 μl of Propidium Iodide (10 mg/ml) and 1 μl of Ribonuclease A. The staining was carried out for at least 3 h at 4 °C. The samples were analysed using the EXPO32ADCXL4 Colour program on a Beckman Coulter Epics XL-MCL machine.

IF staining

Cells grown on coverslips were fixed in 4% formaldehyde for 10 min at 37 °C and permeabilised using 0.5% NP40, 0.3 mM sucrose in PBS. Slides were blocked in 10% FCS in PBS + 0.1% Tween. E2 was detected using the TVG261 mouse monoclonal E2 antibody at 1:5 dilution and anti-mouse-FITC conjugated antibody (Sigma) at 1:1000

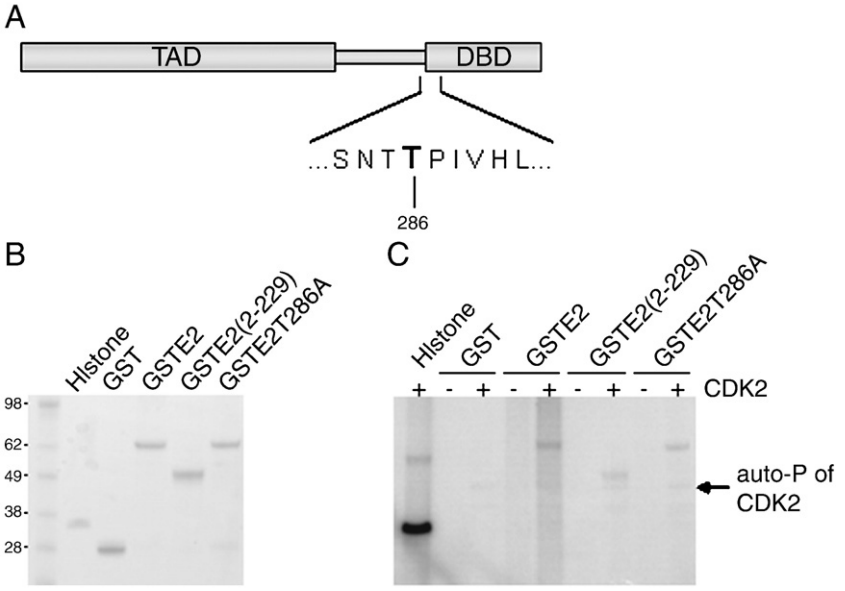

Fig. 5. Cdk2 phosphorylates E2 *in vitro*. (A) There is a consensus Cdk2 phosphorylation site at residue threonine 286 as shown. (B) GST fusion proteins were prepared from *E. coli* and run on an SDS-PAGE gel and stained with Coomassie Brilliant Blue. (C) *In vitro* Cdk2 kinase assays were carried out with recombinant Cdk2 and the GST fusion proteins; histone H1 was included as a positive control as it is a known target protein for Cdk2 mediated phosphorylation. The arrow shows the autophosphorylation of the Cdk2 kinase.

dilution. Both antibodies were diluted in PBS + 0.1% Tween containing 1% FCS. Slides were mounted using Vectashield containing DAPI. Cells were examined with a Leica TCS SP2 confocal microscope and the Leica Confocal Software.

Western blot

Cells were lysed in NP40 lysis buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.5% NP40). For salt fractionation, cell pellets were serially extracted in the same buffer using salt concentrations ranging from 0.1 to 1 M. Protein concentration was determined using the BCA assay (Sigma). Equal amounts (30–50 μg) of total protein were loaded on a 4–12% gradient gel (Invitrogen) and run for 1 h. Proteins were transferred to a nitrocellulose membrane with Invitrogen's iBlot system. Membrane was blocked in 5% milk in PBS + 0.05% Tween. E2 and γ-tubulin were detected using the TVG261 (Hibma et al., 1995) and GTU88 monoclonal antibodies, respectively. Western blots were scanned and quantified using the Image J software. The antibody Mab 96 against SF2/ASF was a gift from Dr. Javier Caceres, Edinburgh.

Real-time qPCR

RNA was prepared using the Trizol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesised from 1–2 μg of total RNA using Superscript II (Invitrogen) and an oligo-dT primer. E2 and β-actin RNA levels were detected using real-time qPCR (probe and primer sets are available upon request).

Phosphoprotein purification

Phosphoprotein purification from synchronised cells was carried out using the Phosphoprotein Purification Kit (Qiagen) according to the manufacturer's instructions. The change in E2 levels was verified in samples from the same treated cell population prepared with buffer provided in the kit as well as NP40 lysis buffer. The lysates were loaded onto columns, eluted, TCA precipitated and western blotted for E2.

Site-directed mutagenesis

Mutation of the E2 sequence encoding threonine 286 to alanine was carried out using the KOD Hot Start DNA polymerase (Novagen) according to Novagen's protocol for site-directed mutagenesis.

GST protein expression

To purify GST proteins, bacteria induced with 2 mM IPTG were lysed in NETN buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40) and sonicated in an ice water bath for 12 × 30 s. The cleared lysate was incubated with Glutathione sepharose (GE Healthcare) for 1.5 h and the beads were washed five times in NETN buffer. The amount of GST fusion protein was estimated on a SDS-PAGE gel stained with Coomassie Brilliant Blue.

Cdk2 phosphorylation assays

Fifty nanograms of recombinant purified human CDK2/CyclinA (Invitrogen) and 2 μg of GST protein or histones (Sigma) were preincubated at 30 °C for 10 min. Kinase buffer (20 mM Tris pH 7.5, 5 mM $MgCl_2$, 100 mM ATP, 0.5 μCi ^{32}P-γATP) was added and samples were incubated for an additional 10 min at 30 °C. Samples were run on an SDS-PAGE gel and phosphorylated proteins were identified using a phosphorimager.

Acknowledgments

We thank Wellbeing of Women for funding this project and Dr. Lubna Nasir and Professor Saveria Campo for critical reading of the manuscript. We also thank Dr. Mary Donaldson for assistance with flow cytometry.

References

Aleem, E., Berthet, C., Kaldis, P., 2004. Cdk2 as a master of S phase entry: fact or fake? Cell. Cycle 3, 35–37.
Bellanger, S., Demeret, C., Goyat, S., Thierry, F., 2001. Stability of the human papillomavirus type 18 E2 protein is regulated by a proteasome degradation pathway through its amino-terminal transactivation domain. J. Virol. 75, 7244–7251.
Benson, J.D., Howley, P.M., 1995. Amino-terminal domains of the bovine papillomavirus type 1 E1 and E2 proteins participate in complex formation. J. Virol. 69, 4364–4372.
Boner, W., Taylor, E.R., Tsirimonaki, E., Yamane, K., Campo, M.S., Morgan, I.M., 2002. A Functional Interaction between the Human Papillomavirus 16 Transcription/Replication Factor E2 and the DNA Damage Response Protein TopBP1. J. Biol. Chem. 277, 22297–22303.
Bouvard, V., Storey, A., Pim, D., Banks, L., 1994. Characterization of the human papillomavirus E2 protein: evidence of trans-activation and trans-repression in cervical keratinocytes. EMBO J. 13, 5451–5459.
Donaldson, M.M., Boner, W., Morgan, I.M., 2007. TopBP1 regulates human papillomavirus type 16 E2 interaction with chromatin. J. Virol. 81, 4338–4342.
Hanamura, A., Caceres, J.F., Mayeda, A., Franza, B.R., Krainer, A.R., 1998. Regulated tissue-specific expression of antagonistic pre-mRNA splicing factors. RNA 4, 430–444.
Hibma, M.H., Raj, K., Ely, S.J., Stanley, M., Crawford, L., 1995. The interaction between human papillomavirus type 16 E1 and E2 proteins is blocked by an antibody to the N-terminal region of E2. Eur. J. Biochem. 229, 517–525.
Hu, Y., Clower, R.V., Melendy, T., 2006. Cellular topoisomerase I modulates origin binding by bovine papillomavirus type 1 E1. J. Virol. 80, 4363–4371.
Loo, Y.M., Melendy, T., 2004. Recruitment of replication protein A by the papillomavirus E1 protein and modulation by single-stranded DNA. J. Virol. 78, 1605–1615.
Ma, T., Zou, N., Lin, B.Y., Chow, L.T., Harper, J.W., 1999. Interaction between cyclin-dependent kinases and human papillomavirus replication-initiation protein E1 is required for efficient viral replication. Proc. Natl. Acad. Sci. U. S. A. 96, 382–387.
Masterson, P.J., Stanley, M.A., Lewis, A.P., Romanos, M.A., 1998. A C-terminal helicase domain of the human papillomavirus E1 protein binds E2 and the DNA polymerase alpha-primase p68 subunit. J. Virol. 72, 7407–7419.
McPhillips, M.G., Oliveira, J.G., Spindler, J.E., Mitra, R., McBride, A.A., 2006. Brd4 is required for e2-mediated transcriptional activation but not genome partitioning of all papillomaviruses. J. Virol. 80, 9530–9543.
Mohr, I.J., Clark, R., Sun, S., Androphy, E.J., MacPherson, P., Botchan, M.R., 1990. Targeting the E1 replication protein to the papillomavirus origin of replication by complex formation with the E2 transactivator. Science 250, 1694–1699.
Parish, J.L., Bean, A.M., Park, R.B., Androphy, E.J., 2006. ChlR1 is required for loading papillomavirus E2 onto mitotic chromosomes and viral genome maintenance. Mol. Cell. 24, 867–876.
Penrose, K.J., McBride, A.A., 2000. Proteasome-mediated degradation of the papillomavirus E2-TA protein is regulated by phosphorylation and can modulate viral genome copy number. J. Virol. 74, 6031–6038.
Penrose, K.J., Garcia-Alai, M., Prat-Gay, G., McBride, A.A., 2004. Casein Kinase II phosphorylation-induced conformational switch triggers degradation of the papillomavirus E2 protein. J. Biol. Chem. 279, 22430–22439.
Schuck, S., Stenlund, A., 2005. Assembly of a double hexameric helicase. Mol. Cell 20, 377–389.
Steger, G., Ham, J., Yaniv, M., 1996. E2 proteins: modulators of papillomavirus transcription and replication. Methods Enzymol. 274, 173–185.
Steger, G., Corbach, S., 1997. Dose-dependent regulation of the early promoter of human papillomavirus type 18 by the viral E2 protein. J. Virol. 71, 50–58.
Taylor, E.R., Boner, W., Dornan, E.S., Corr, E.M., Morgan, I.M., 2003. UVB irradiation reduces the half-life and transactivation potential of the human papillomavirus 16 E2 protein. Oncogene 22, 4469–4477.
Van Tine, B.A., Dao, L.D., Wu, S.Y., Sonbuchner, T.M., Lin, B.Y., Zou, N., Chiang, C.M., Broker, T.R., Chow, L.T., 2004. Human papillomavirus (HPV) origin-binding protein associates with mitotic spindles to enable viral DNA partitioning. Proc. Natl. Acad. Sci. U. S. A. 101, 4030–4035.
Wu, Y.C., Bian, X.L., Heaton, P.R., Deyrieux, A.F., Wilson, V.G., 2009. Host cell sumoylation level influences papillomavirus E2 protein stability. Virology 387, 176–183.
You, J., Croyle, J.L., Nishimura, A., Ozato, K., Howley, P.M., 2004. Interaction of the bovine papillomavirus E2 protein with Brd4 tethers the viral DNA to host mitotic chromosomes. Cell 117, 349–360.
Yu, J.H., Lin, B.Y., Deng, W., Broker, T.R., Chow, L.T., 2007. Mitogen-activated protein kinases activate the nuclear localization sequence of human papillomavirus type 11 E1 DNA helicase to promote efficient nuclear import. J. Virol. 81, 5066–5078.