



Interaction between the HPV-16 E2 transcriptional activator and p53

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The HPV-16 E2 protein is a major regulator of viral DNA replication and gene expression. Through interactions with the viral origin binding protein, E1, it localizes E1 to the origin of replication and stimulates the initiation of viral DNA replication. However, several recent reports have described a number of diverse activities of E2 relating to the induction of apoptosis through both p53 dependent and independent mechanisms, and to induction of growth arrest in both the G1 and G2M phases of the cell cycle. Recent studies have also shown that p53 can specifically inhibit HPV DNA replication, albeit through an unknown mechanism. Since p53 has been described in the replication centres of Herpes Viruses, Adenovirus and SV40 we decided to investigate whether any of the above activities of E2 may be related to an association with p53. We show, in a series of *in vitro* assays, specific interaction between p53 and HPV-16 E2 via residues in the carboxy terminal half of the E2 protein. Mutational analysis of p53 indicates that sequences in both the DNA binding and oligomerization domains are essential for the interaction, and a mutant of p53 which is unable to bind E2 is also unable to inhibit HPV DNA replication. Finally, using an inducible system of p53 expression we also show that E2 will complex with p53 *in vivo*. These results raise the intriguing possibility that p53 may also be involved in HPV DNA replication centres, and also provides explanations for some of the diverse activities reported for the HPV E2 proteins.

Keywords: HPV; E2; p53

Introduction

The HPV-16 E2 protein is a major regulator of viral gene expression and DNA replication. The protein has a modular structure comprising a transactivation domain, a flexible hinge region, and a carboxy terminal domain which is important for dimerization and DNA binding activity (Giri and Yaniv, 1988). Within the HPV-16 long control region (LCR) there are four specific E2 recognition sites, characterized by the sequence ACCGN4CGGT, to which E2 binds as a dimer (Androphy *et al.*, 1987a). Through interactions at these sites E2 can specifically upregulate the viral oncogene expression (Cripe *et al.*, 1987; Bouvard *et al.*, 1994; Kovelman *et al.*, 1996). Under certain circum-

stances, when the E2 protein is overexpressed, transcriptional repression is also observed (Bouvard *et al.*, 1994; Steger and Corbach, 1997). Two of the E2 binding sites in the LCR are located close to the viral origin (ori) of DNA replication (Remm *et al.*, 1992; Lu *et al.*, 1993). Through its ability to interact with the viral ori binding protein E1, E2 brings about a local concentration of E1 at the ori, and thus stimulates the initiation of viral DNA replication (Sedman and Stenlund, 1995, 1996). Extensive mutational analyses have now clearly separated the functions of E2 important for transcriptional activation, and for stimulation of viral DNA replication (Sakai *et al.*, 1996; Piccini *et al.*, 1997).

As well as these obvious virological activities of the E2 protein, there are a number of reports of additional functions of E2. Overexpression of E2 has been reported to induce apoptosis in a number of different assay systems. Some of this activity is related to the ability of E2 to repress viral gene expression when it is highly expressed, resulting in a decrease in E6 expression and consequent increased levels of p53 (Desaintes *et al.*, 1997). However, there are other examples of E2 inducing apoptosis through mechanisms which are still p53 dependent, but are unrelated to its effects on E6 expression (Desaintes *et al.*, 1997). In addition, E2 has also been shown to induce growth arrest in the G1 phase of the cell cycle (Hwang *et al.*, 1996; Goodwin *et al.*, 1998), as well as to abrogate the mitotic checkpoint (Fratini *et al.*, 1997) through both p53 dependent and independent mechanisms.

A number of different DNA tumour viruses have now been shown to inhibit the activity of the cellular tumour suppressor protein, p53. These inhibitory activities are frequently attained via quite diverse mechanisms. SV40 large T antigen binds and sequesters p53 (Bargonetti *et al.*, 1992), Adenovirus E1B 55 kd protein binds to, and inactivates, p53 transcriptional activity (Yew *et al.*, 1994), although recent reports indicate that Adenovirus proteins will also target p53 for degradation (Querido *et al.*, 1997; Steegenga *et al.*, 1998). The E6 proteins from the high-risk HPV types are normally expressed at very low levels (Androphy *et al.*, 1987b; Banks *et al.*, 1987) and they overcome p53 function by targeting it for rapid proteasome mediated degradation (Werness *et al.*, 1990; Scheffner *et al.*, 1990). Although these viruses have developed elaborate mechanisms for overcoming the growth inhibitory functions of p53, there are now several reports of p53 being found within viral DNA replication centres in infected cells. These were first described for SV40 (Braithwaite *et al.*, 1987; Gannon and Lane, 1987), but p53 was also subsequently shown to be present in the DNA replication compartments of Herpes simplex (HSV) and also Cytomegalovirus

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(CMV) infected cells (Wilcock and Lane, 1991; Zhong and Hayward, 1997; Fortunato and Spector, 1998). p53 has also recently been reported in viral DNA replication centres in Adenovirus infected cells (Konig *et al.*, 1999). The potential role of p53 in HPV DNA replication has not been extensively addressed, although a recent study showed that p53 could specifically inhibit HPV DNA replication (Lepik *et al.*, 1998). We have been interested in whether p53 may exert this activity by interacting with a component of the HPV DNA replication machinery. Taking into account the pleiotropic activities which have been reported for E2, it was natural to investigate whether or not E2 might interact with p53. We show both *in vitro* and *in vivo* that HPV-16 E2 specifically interacts with the p53 protein. This interaction requires sequences in the p53 DNA binding and oligomerization domains and sequences within the carboxy terminus of E2. Further, a mutant of p53 which cannot bind E2 is defective in its ability to inhibit viral DNA replication. These results demonstrate that p53 may play a role in HPV DNA replication, and suggest the need for caution in interpreting E2 biological activity in situations of overexpression.

Results

HPV-16 E2 binds p53 through sequences in its carboxy terminus

In order to investigate the possibility of an interaction between HPV-16 E2 and p53, fragments of E2 were expressed in *E. coli* as GST fusion proteins encoding full length, amino terminal (amino acids 1–138) and carboxy terminal sequences (amino acids 237–365). The GST fusion proteins were purified and their ability to bind p53 was assessed by performing a series of GST pull-down assays with *in vitro* translated radiolabelled p53. The results obtained are shown in Figure 1. It is clear that full length HPV-16 E2 retains a significant proportion of the input p53 protein compared with no binding on the GST control. Interestingly, when the binding assay is performed with the amino and carboxy terminal portions of the E2 protein, it shows that all the sequences necessary for binding p53 lie within the carboxy terminal half of the E2 protein. No interaction was observed with the amino terminal sequences of the E2 protein.

To further define the region of E2 responsible for the interaction with p53 a series of truncated GST-E2 fusion proteins were produced. These are shown schematically in Figure 2a. The fusion proteins were again purified, and binding assays were performed with *in vitro* translated p53; the results obtained are shown in Figure 2b. As can be seen, wild type levels of binding to p53 are seen with the fusion protein containing sequences from 250–351. Further truncations result in a progressive loss of binding to p53 with the 250–307 construct being completely negative. These results indicate that the region of E2 essential for binding to p53 lies predominantly within the sequence 339–351. This region of E2 contains sequences necessary for dimerization but it lies outside the core DNA binding domain of the protein (Prakash *et al.*, 1992; Hedge *et al.*, 1992).

P53 binding to E2 correlates with its ability to inhibit HPV DNA replication

Having shown that HPV-16 E2 can bind to p53 through a small stretch of amino acids within its carboxy terminal region, we were next interested in defining which regions of p53 were necessary for complex formation with E2. To do this, a series of binding assays were performed with GST-E2 fusion protein and mutants of p53 previously shown to be defective in oligomerization (Tarunina and Jenkins, 1993). The results obtained are shown in Figure 3. It is clear that wild type p53 is again retained with a high efficiency, as is the mutant H175. This is a naturally occurring mutant of p53 and is defective in its ability to bind the consensus DNA recognition sequence. In contrast, the carboxy terminal mutants of p53, defective for oligomerization, were all reduced in their ability to bind E2. The 518 mutant, which only forms dimers, showed weak binding to E2, but the 338 and 1262 mutants, both of which can only exist as monomers, failed to bind to E2. These results indicate that sequences within the carboxy terminus of p53 contribute to the ability of p53 to bind to HPV-16 E2.

To further investigate whether the carboxy terminal sequences of p53 were directly responsible for binding E2 or whether reduced binding was a consequence of the loss of oligomerization, we performed a series of binding assays in which a monomeric mutant of p53 was restored to a dimer through incubation with an amino terminal monoclonal antibody, similar to studies performed previously (Thomas *et al.*, 1996). GST-E2 fusion protein was purified as above and incubated with either *in vitro* translated wild type p53 or the monomeric mutant 338. The results obtained are shown in Figure 4. As can be seen in the presence of preimmune antibody the 338 mutant failed to bind to the GST-E2 fusion protein. In contrast, if the *in vitro*

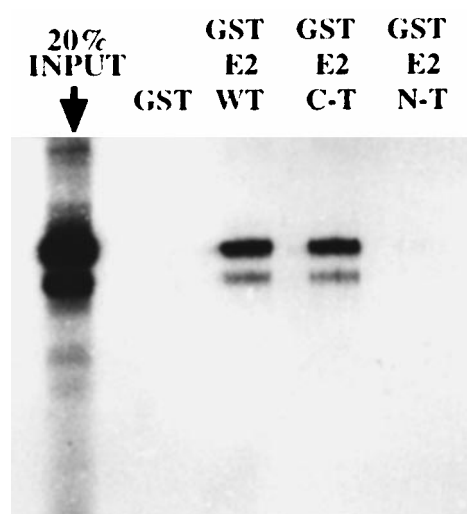


Figure 1 HPV-16 E2 binds p53 through sequences in its carboxy terminus. HPV-16 E2 was expressed as GST fusion proteins encoding either full length E2 (WT), carboxy terminal sequences from 237–365 (C-T) or amino terminal sequences from 1–138 (N-T), purified and equal amounts used to measure binding to *in vitro* translated radiolabelled p53. The bound p53 was assessed by PAGE and autoradiography. Input p53 and binding to control GST protein is also shown

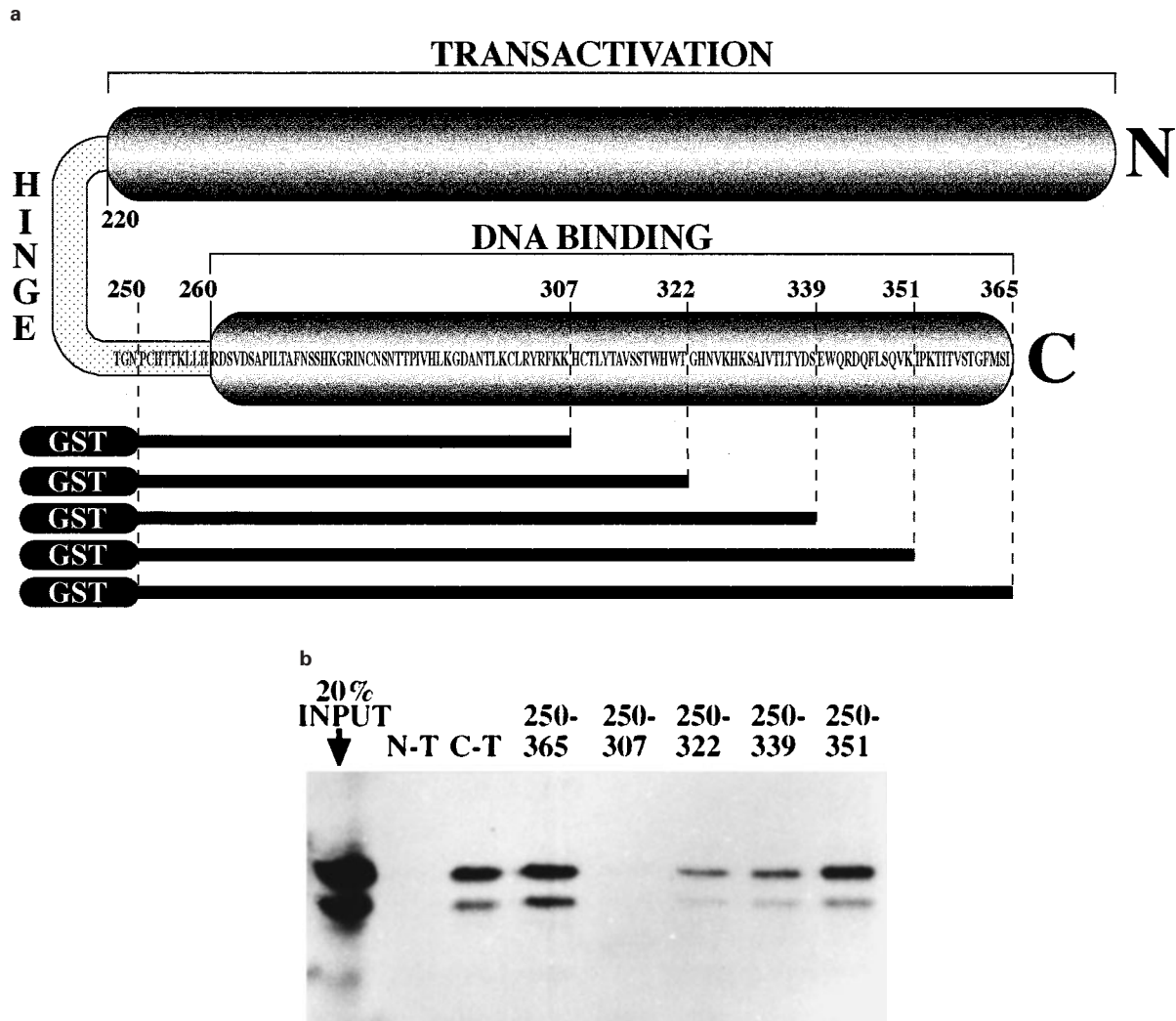


Figure 2 Identification of the minimal region of E2 binding to p53. (a) Schematic diagram showing the different GST-E2 fusion proteins which were constructed in order to identify which region of the carboxy terminus of E2 was responsible for binding p53; (b) an *in vitro* binding assay was performed as in Figure 1 using the GST-E2 fusion proteins as indicated. Bound p53 was determined following PAGE and autoradiography and input p53 is also shown

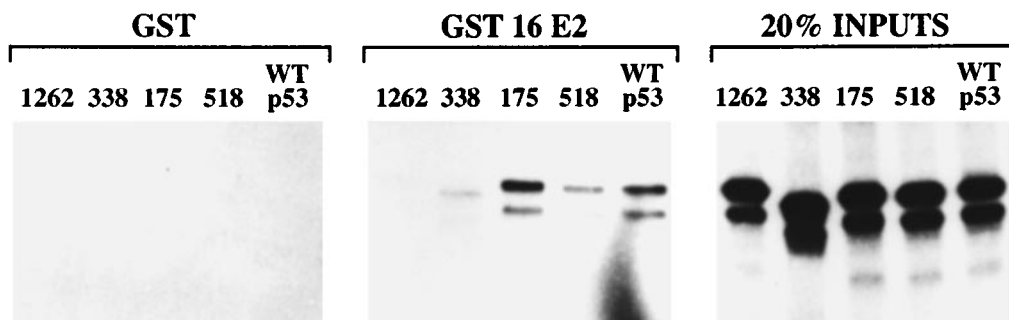


Figure 3 Sequences within the carboxy terminus of p53 are required for binding E2. The ability of the indicated mutants of p53 to bind E2 was assessed by performing GST pull-down assays with GST or wild type GST-E2 fusion protein. The bound p53 was determined following PAGE and autoradiography and the inputs of the p53 proteins are shown

translated p53 protein was pre-incubated in the presence of pAb1801, which binds sequences within the amino terminal region of p53 (Banks *et al.*, 1986), the ability of 338 to bind E2 is restored. When the same assay was performed using pAb1802, which binds sequences in the carboxy terminus of p53 that are absent in the 338 mutant (Banks *et al.*, 1986), no effect on the ability of 338 to bind E2 was observed. These

results demonstrate that efficient interaction between E2 and p53 requires p53 oligomerization and, further, that the sequences in the carboxy terminal region of p53 are not directly involved in the association.

Previous studies had shown that the trp248 mutant of p53, which lies close to the H175 mutant used in this study, was defective in its ability to inhibit HPV DNA replication (Lepik *et al.*, 1998). We therefore investi-

gated the ability of the trp248 mutant to bind HPV-16 E2 and the results obtained are shown in Figure 5. As can be seen, this mutant is greatly reduced in its ability to interact with HPV-16 E2, when compared to the wild type p53. In addition, the presence of pAb1801 has no stimulatory effect upon its ability to bind E2, thus further demonstrating the specificity of the results shown in Figure 4.

Having defined the regions of p53 which are important for binding E2, we next analysed the capacity of these mutant p53 proteins to inhibit HPV DNA replication *in vivo*. Human 293 cells were transfected with an HPV-16 ori containing plasmid, together with a plasmid expressing the HPV-16 E1 and E2 proteins plus the different p53 mutants. The cells were harvested after 72 h and the amount of replicated plasmid DNA was assessed following *DpnI* digestion and Southern blot analysis. The results obtained are shown in Figure 6. In agreement with previous observations, the wild type p53 strongly inhibits HPV DNA replication (Lepik *et al.*, 1998). Interestingly the trp248 mutation which is defective for E2 binding, is also defective in its ability to inhibit HPV replication. This contrasts with the H175 mutant which binds E2 with wild type ability and is also wild type with respect to its ability to inhibit viral DNA replication. However, the oligomerization defective mutants of

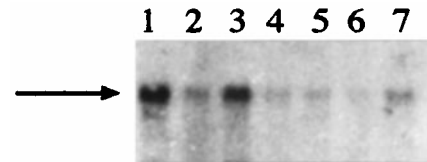


Figure 6 Abolition of transient HPV DNA replication by p53. Human 293 cells were transfected with an HPV-16 ori containing plasmid together with an HPV-16 E1/E2 expressing plasmid together with wild type and mutant derivative of p53. After 72 h the cells were extracted, DNA digested with *DpnI* and replicated DNA detected following Southern blot hybridization. The arrow indicates the replication product. All lanes contain HPV ori and E1/E2 expression plasmids; the remaining lane compositions are: lane 1 vector alone; lane 2 wild type p53; lane 3 trp248; lane 4 dimeric mutant 518; lane 5 monomeric mutant 1262; lane 6 monomeric mutant 338; lane 7 H175

p53 were also equally effective at inhibiting HPV DNA replication. This would appear to rule out an involvement of E2 in this activity of p53. Nonetheless, these mutants have all the necessary sequences for binding E2, once they assume a higher order structure (see Figure 4), which is certainly not the case with the trp248 mutation.

HPV-16 E2 binds p53 *in vivo*

Having shown that HPV-16 E2 will complex with p53 *in vitro*, we were next interested in ascertaining whether this interaction could also take place *in vivo*. To do this we made use of a cell line that constitutively expresses p53, whose function is inducible (Littlewood *et al.*, 1995). p53 was fused to a mutant form of the oestrogen receptor (ER) and stable cell lines were grown out following drug selection. The function of the p53-ER fusion protein could then be induced following treatment with 4-hydroxy tamoxifen as previously described (Littlewood *et al.*, 1995). Figure 7a shows the levels of p53 fusion protein expression in uninduced cells and 6 h post induction compared with control transfected Saos-2 cells. Addition of inducer does not significantly affect p53-ER levels over this period of time. We then used these cells to ascertain whether HPV-16 E2 could complex with p53 *in vivo*. The p53 expressing cells were transfected with a plasmid expressing high levels of HPV-16 E2 (Bouvard *et al.*, 1994) and after 18 h p53 function was induced in some transfectants by the addition of ligand. After a further 6 h the cells were harvested and E2 protein immunoprecipitated with an anti-E2 polyclonal antibody. The precipitates were then Western blotted and co-immunoprecipitated p53 was detected by probing with a pool of anti-p53 monoclonal antibodies (Banks *et al.*, 1986). The results obtained are shown in Figure 7b. In cells transfected with E2 expression plasmid, no co-immunoprecipitating p53 was seen when the p53 was not induced, nor if the immunoprecipitation was performed with a preimmune antibody. In addition, in the absence of E2 expression plasmid the anti-E2 antibody only co-immunoprecipitated minimal levels of p53 protein. In contrast, in cells which express E2 and in which the p53 protein is induced, a significant amount of p53 is co-immunoprecipitated with the anti E2 antibody. These results demonstrate conclusively that HPV-16 E2 and p53 will indeed form complexes *in vivo*.

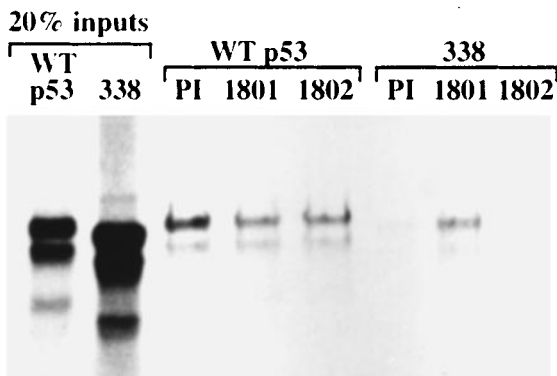


Figure 4 p53 oligomerization is essential for binding E2. The ability of wild type p53 (WT) and the 338 mutant to bind GST-E2 was assessed following pre-incubation for 30 min with preimmune antibody (PI) or the p53 specific monoclonal antibodies pAb1801 or pAb1802 as indicated: the 338 mutant lacks the 1802 epitope. The bound p53 was determined following PAGE and autoradiography and inputs of the p53 proteins are shown

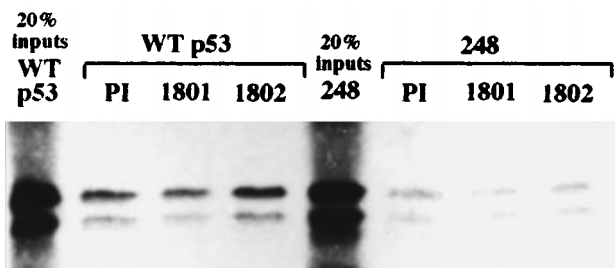


Figure 5 The trp248 mutation abolishes E2 binding. The ability of wild type (WT) and the trp248 (248) mutant to bind GST-E2 was assessed following pre-incubation with preimmune (PI) or the p53 specific monoclonal antibodies pAb1801 and pAb1802. The bound p53 was determined following PAGE and autoradiography and inputs of the p53 proteins are shown

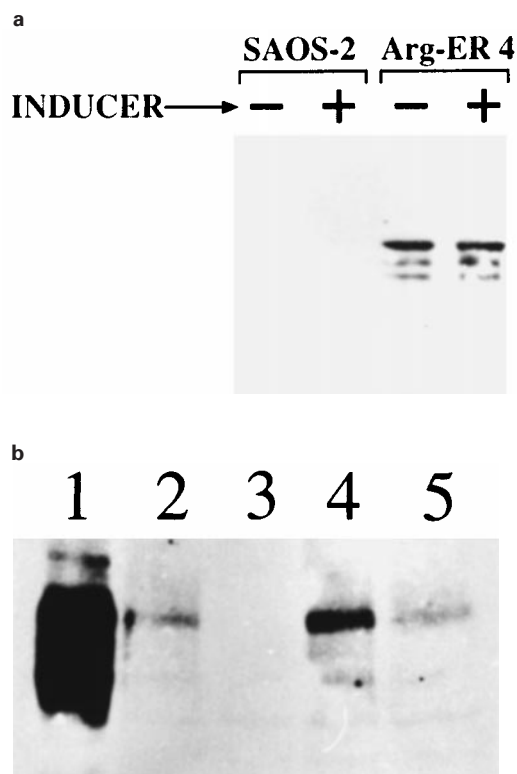


Figure 7 HPV-16 E2 binds p53 *in vivo*. (a) Levels of ER-p53 expression in control Saos-2 cells and ER-p53 cells. Cells were grown in the presence or absence of 4-hydroxytamoxifen for 6 h, extracted and p53 levels ascertained by Western blotting and detection with a pool of anti p53 monoclonal antibodies; (b) Saos-2 cells stably expressing an ER-p53 fusion protein were transfected with either an E2 expression plasmid (lanes 3–5) or vector alone (lane 2). After 18 h, the p53 protein was either induced with 4-hydroxytamoxifen (lanes 2–4) for 6 h or left uninduced (lane 5). Cells were then harvested and immunoprecipitated with either anti E2 antibody (lanes 2, 4 and 5) or preimmune antibody (lane 3). Coprecipitated p53 was detected by Western blotting using a pool of anti p53 monoclonal antibodies as probes. Lane 1 shows 10% of the extract used for the immunoprecipitations

Discussion

The HPV-16 E2 protein is a major regulator of viral gene expression and has a number of additional pleiotropic effects within the cell. These include the induction of apoptosis and growth arrest in G1 and abrogation of the mitotic checkpoint control during the cell cycle. These activities appear to be mediated by both p53 dependent and independent pathways (Hwang *et al.*, 1996; Goodwin *et al.*, 1996; Desaintes *et al.*, 1997; Frattini *et al.*, 1997). Since p53 has also been reported to specifically inhibit HPV DNA replication, we performed a series of studies to investigate any possible associations between E2 and p53. We show in a series of assays, performed both *in vitro* and *in vivo*, that HPV-16 E2 and p53 are capable of forming protein–protein interactions. These results provide a number of possible explanations for the diverse activities reported for the HPV-16 E2 protein.

We first investigated the potential for an E2–p53 interaction using a series of GST-E2 fusion proteins and *in vitro* translated p53. It is clear that all the sequences within E2 that are necessary for binding p53 lie within the carboxy terminal portion of the protein.

More detailed deletion analysis of the HPV-16 E2 protein defined the minimal region responsible for binding p53 as spanning amino acids 339–351, although sequences from 307–339 would also appear to contribute to the interaction. We cannot formally exclude any involvement of the region spanning residues 250–307, but this seems unlikely. Residues 339–351 lie outside the region of E2 which is responsible for DNA binding, and this excludes the possibility that the interaction is mediated via non specific interaction through any residual DNA. However, this region of E2 does contain sequences necessary for E2 dimerization and it will be of interest to determine whether this aspect of E2 function is affected in the presence of p53.

Mutational analysis of p53 revealed a number of interesting points. Firstly, mutants which affected p53 oligomerization were also defective for binding E2. In contrast, the DNA binding-defective H175 mutant of p53 retained the ability to bind E2 whereas the trp248 mutant, which is also defective for DNA binding, did not. We therefore compared the ability of these two mutants to inhibit viral DNA replication and found that, whereas the trp248 was defective, and this is in agreement with previous studies (Lepik *et al.*, 1998), the H175 exhibited wild type levels of activity. Therefore these studies would tend support the hypothesis that p53 inhibits HPV DNA replication through an interaction with the viral E2 protein. However the oligomerization defective mutants of p53, although defective for binding E2, nonetheless still retain the ability to inhibit viral DNA replication. The most likely explanation for this apparent contradiction comes from the studies which showed that sequences within the carboxy terminal region of p53 are not directly involved in the association with E2. Thus, in a series of studies with the monomeric mutant of p53, 338, which is defective in its ability to bind E2, prior incubation with a monoclonal antibody which induces dimerization, partially restored the ability of this mutant to bind E2. These results suggest, therefore, that p53 oligomerization is essential for complex formation with E2 *in vitro*, but that the sequences within the p53 protein which are directly involved lie outside this carboxy terminal domain. At present we have no information as to what proportion of the 338 and 1262 mutants of p53 are present in higher order complexes within the cell, suffice to say, that previous studies have shown that these mutants are capable of activating target promoter expression (Thomas *et al.*, 1996), indicating residual ability to form multimeric complexes.

A key aspect in defining any protein–protein interaction is the ability to demonstrate its existence *in vivo*. To do this we have made use of a novel system, in which p53 expression is constitutive, but its function is inducible. This enables us to attain high levels of p53 expression without obtaining the deleterious effects on cell survival which would normally be encountered. By transfecting a HPV-16 E2 expression plasmid into these cells, followed by immunoprecipitation of E2 and p53 detection by Western blotting, we have been able to demonstrate that the E2–p53 association also takes place *in vivo*. An important aspect of this particular assay was the lack of association between E2 and p53 when the p53 protein function was not induced. In the

absence of ligand the p53-ER fusion protein is present as an inactive complex with HSP90 and other polypeptides (Pratt, 1990 for review). This complex would also not appear to be accessible to the HPV-16 E2 protein, since no significant degree of co-immunoprecipitation was obtained under these conditions. However, upon induction of p53, efficient complex formation with the E2 protein could be detected. These results demonstrate that the E2-p53 interaction also takes place *in vivo*.

At present the biological significance of the E2-p53 association is unclear. However, it is intriguing to note that p53 has been found in the viral replication centres of SV40, HSV, CMV and Adenovirus (Braithwaite *et al.*, 1987; Gannon and Lane, 1987; Wilcock and Lane, 1991; Zhang and Hayward, 1997; Fortunato and Spector, 1998; Konig *et al.*, 1999). Whether this represents a means by which p53 is inhibiting viral replication, or whether this represents an actual role for p53 in viral replication remains to be determined. It is interesting to note that a recent report also described HPV DNA replication occurring in very similar nuclear structures to those described for HSV and Adenovirus, although the status of p53 within these complexes was not determined (Swindle *et al.*, 1999). We tend to favour the hypothesis that some p53 may, in fact, not be deleterious to all viral replication, since in the case of HPV an elaborate mechanism appears to have evolved, involving alternative splicing within the E6 open reading frame which fine-tunes the levels of p53 present within the cell (Pim *et al.*, 1997). Studies are in progress to investigate these aspects further.

The E2-p53 association may also be of relevance for some of the additional known activities of E2, unrelated to viral gene expression and DNA replication. A number of these activities, in particular induction of apoptosis and G1 arrest, have been assigned to pathways which are p53 dependent but which are independent of E2's ability to regulate viral gene expression. Obviously, both the apoptotic and growth arrest pathways are regulated directly by p53 and it is not inconceivable that some of these activities of E2 might be mediated through an association with p53. Considering the importance of p53 in regulating a large number of different pathways within the cell, there is an obvious need for caution in interpreting the results of studies in which the E2 proteins are over-expressed.

In conclusion, we have shown a specific and efficient interaction between the HPV-16 E2 protein and p53. These data have potentially important implications both for viral pathogenesis and for elucidation of the mechanisms of action of the HPV E2 proteins.

Materials and methods

Cells and transfections

Saos-2 cells were grown in DMEM supplemented with 10% foetal calf serum. Transfections were performed using calcium phosphate precipitation as described previously (Matlaszewski *et al.*, 1987).

GST fusion protein purification and *in vitro* binding assays

GST fusion protein expression and purification was done as described previously (Thomas *et al.*, 1996). *In vitro* translations were performed using the TNT coupled reticulocyte lysate system (Promega). The GST-E2, GST-E2CT and GST-E2NT constructs have been described previously (Piccini *et al.*, 1995) and the series of E2 carboxy terminal truncations were generated using PCR amplification and were verified by DNA sequencing. The wild type and mutant p53 expression constructs have also been described previously (Tarunina and Jenkins, 1993; Thomas *et al.*, 1996). Briefly, the dimeric mutant 518 has point mutations 373L, 380L and 387L; monomeric mutant 1262 has point mutations 341K, 344E, 348E and 355K and monomeric mutant 338 is truncated at amino acid residue 338. The trp248 mutant was kindly provided by Bert Vogelstein.

Transient DNA replication assay

These were performed in human 293 cells and were processed as described previously (Piccini *et al.*, 1997).

Generation of p53Arg-ER 4, a cell line constitutively expressing a p53-ER fusion protein

The construct pCDNA3.1 p53Arg-ER was generated by PCR cloning the p53-ER sequences from construct pBabepuro p53ERTM (kind gift of Gerard Evan) and inserting them into the *HindIII/EcoRI* sites of plasmid pCDNA3.1 zeo (Invitrogen). The new construct contains the open reading frame of wild type p53 (Arginine polymorphism at residue 72) fused to sequences encoding the hormone-binding domain of a mutant murine oestrogen receptor (Danielian *et al.*, 1993). The construct was transfected into Saos-2 osteosarcoma cells, and the cells placed under Zeocin (Invitrogen) selection. Drug-resistant colonies were picked, grown up and tested for constitutive expression of the p53-ER fusion protein by Western blot analysis. The cell line p53Arg-ER 4 generated by this method constitutively expresses the p53Arg-ER fusion protein, whose function is then inducible by addition of the synthetic ligand, 4-hydroxytamoxifen, to the cell medium at a concentration of 100 nM.

In vivo co-immunoprecipitation

The p53Arg-ER 4 cells were transfected either with pCMV-E2 (Bouvard *et al.*, 1994) or with empty vector. After 18 h cells were either induced or uninduced with 4-hydroxytamoxifen, and proteins extracted as described previously (Thomas *et al.*, 1996). Extracts were immunoprecipitated with either an anti-E2 polyclonal antibody (Piccini *et al.* 1997) or pre-immune antibody. Precipitates were then subjected to SDS-PAGE and transferred to nitrocellulose. Co-precipitated p53 was detected by using a pool of the anti-p53 monoclonal antibodies pAb1801, pAb1802 and pAb1803 (Banks *et al.*, 1986) and developed using the Amersham ECL according to the manufacturer's instructions.

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