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The Bovine Papillomavirus Type 4 E8 Protein Binds to Ductin and Causes Loss of Gap Junctional Intercellular Communication in Primary Fibroblasts

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The E8 open reading frame of bovine papillomavirus type 4 encodes a small hydrophobic polypeptide which contributes to cell transformation by conferring anchorage-independent growth. Using an in vitro translation system, we show that the E8 polypeptide binds to ductin, the 16-kDa proteolipid that forms transmembrane channels in both gap junctions and vacuolar H^+-ATPase. This association is not due to nonspecific hydrophobic interactions. PPA1, a *Saccharomyces cerevisiae* polypeptide homologous (with 25% identity) to ductin, does not complex with E8. Furthermore, E5B, structurally similar to E8 but with no transforming activity, does not form a complex with ductin. Primary bovine fibroblasts expressing E8 show a loss of gap junctional intercellular communication, and it is suggested that this results from the interaction between E8 and ductin.

Bovine papillomavirus type 4 (BPV-4) infects the mucous epithelium of the alimentary canal of cattle and causes papillomas, which can progress to malignancy in animals that feed on bracken fern (4, 16). However, BPV-4 is incapable of transforming primary bovine fibroblasts (PalFs) by itself and requires the cooperation of an activated *ras* gene to morphologically transform PalFs. The partially transformed PalFs have an extended life span and are capable of anchorage-independent growth but are not immortal or tumorigenic in nude mice (15). The transforming genes of BPV-4 are encoded by E7 and E8 open reading frames (ORFs). E7, the major transforming gene of BPV-4, encodes a polypeptide which possesses two Cys-X-X-Cys motifs and a potential $p105^{Rb}$ binding domain (25). The E8 ORF, which contributes to cellular transformation by conferring anchorage independence (25), encodes a small hydrophobic polypeptide similar in its length (42 residues) and amino acid composition to the BPV-1 E5 polypeptide (if not otherwise specified, E5 refers to the BPV-1 polypeptide).

The E5 polypeptide of BPV-1 has previously been shown to bind to a highly conserved integral membrane protein called ductin (13), and other viral oncoproteins such as human papillomavirus type 16 (HPV-16) E5, HPV-6 E5, and human T-cell leukemia virus type 1 $p12^I$ also bind to ductin (6, 12).

Ductin, a polypeptide of 16 kDa, is very hydrophobic and is thought to contain four transmembrane segments arranged as a four-α-helix bundle (8). Ductin is found in two distinct membrane complexes. It is the subunit c or 16-kDa proteolipid of the vacuolar H^+-ATPase (18), a universal transmembrane proton pump of eukaryotes that is responsible for the acidification of cytoplasmic organelles (22). Ductin provides the pathway for proton translocation in the V_0 sector of the vacuolar H^+-ATPase. Ductin is also a major structural component of gap junctions (10), cell surface specializations that form pathways for the intercellular exchange of cytoplasmic ions and small molecules with molecular weights up to ~1,000. The binding of the E5 polypeptide to ductin has led to the suggestion that both of ductin's transport functions might be impaired (11). A defective vacuolar H^+-ATPase in the early endosomal compartment could effect growth factor receptor down-regulation (observed in cells transformed with BPV-1 E5 or HPV-16 E5) (19, 29, 30), and, indeed, cells expressing HPV-16 E5 exhibit inhibition of endosomal acidification (28).

In this study we have developed an in vitro system to examine the binding of ductin to viral oncoproteins. We show that E8 binds to ductin, that this binding is not a nonspecific hydrophobic interaction, and that primary fibroblasts expressing E8 show a loss of gap junctional intercellular communication (GJIC).

Previous studies examining the interaction between ductin and viral oncoproteins (e.g., BPV-1 E5 [14]) have used transient expression in cultured cells. However, ductin can be efficiently inserted into membranes and assembled into complexes in a cell-free system using rabbit reticulocyte lysate supplemented with canine pancreatic rough microsomal membranes (7). Protein-protein interactions can be studied in this cell-free system, which has the advantage that the analysis of binding activities is facilitated as the coexpressed polypeptides accumulate in the same membrane population.

Expression of E5 and E8 in vitro. The in vitro synthesis and membrane insertion of BPV-1 E5 and BPV-4 E8 polypeptides were first characterized. E5 and E8 were modified by the addition of an 11-amino-acid sequence from the influenza virus hemagglutinin (HA), the HA1 epitope, to their amino-terminal region to construct the fusion proteins HAE5 and HAE8.

For T7 promoter-directed in vitro transcription, the HAE8 ORF was subcloned into the *Bam*HI site of the vector pAlter (Promega). The HAE5 ORF (a gift of R. Schlegel and D. J. Goldstein) was cloned into the *Xho*I-*Bam*HI sites of the pRS303 (Stratagene) vector, containing a T7 promoter for in vitro transcription. All plasmids used for in vitro transcription were linearized, phenol-chloroform extracted, and ethanol precipitated. Transcription reactions were performed with an Ambion transcription kit (AMS Biotechnology UK Ltd.). RNAs were synthesized with T7 polymerase, with cap analog included in the reaction. The in vitro-transcribed capped RNAs were purified and used for the in vitro translation. Each reaction was performed at 30°C for 1 h, in nuclease-treated

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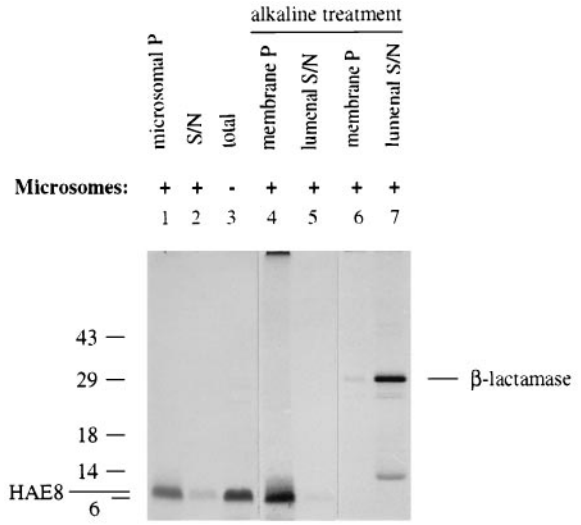

FIG. 1. In vitro expression of E8. The HAE8 protein was translated in the presence of microsomes (+). After centrifugation of the translation reaction mixture, the microsome pellet (P) fraction (lane 1) was separated from the supernatant (S/N) fraction (lane 2). The HAE8 protein was also translated in the absence of microsomes (−) (lane 3). The in vitro-synthesized proteins were immunoprecipitated with 12CA5 monoclonal antibody, which recognizes the HA1 epitope, and separated by SDS-PAGE (14% polyacrylamide). Treatment of the microsome pellet fraction with sodium carbonate, followed by centrifugation, separated an HAE8 membrane pellet fraction (lane 4) from an HAE8 luminal supernatant fraction (lane 5). The control, β-lactamase, was released by sodium carbonate extraction from the lumen to the supernatant fraction (lanes 6 and 7). Molecular masses (in kilodaltons) are indicated at the left.

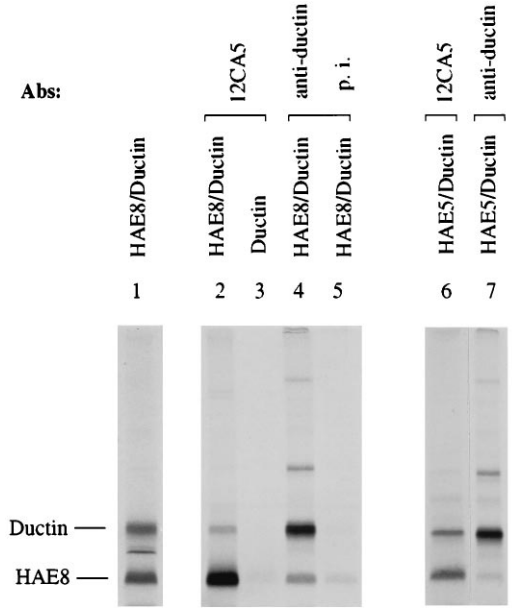

FIG. 2. Association of ductin with E8 protein. In vitro cotranslation in the presence of microsomal membranes of ductin and HAE8, the E8 fusion protein tagged with the HA1 epitope at the amino terminus, is shown in lane 1. Immunoprecipitation of the microsomal membrane pellet fraction using 12CA5 monoclonal antibody raised against the HA1 epitope (lane 2) and an antiserum (polyclonal N2) against ductin (lane 4) are also shown. The immunoprecipitated proteins were separated by SDS-PAGE (14% polyacrylamide) and visualized by autoradiography. Both ductin and E8 proteins are coprecipitated by both antibodies. The controls included immunoprecipitation of ductin using 12CA5 monoclonal antibody (lane 3) and translation of ductin and E8, immunoprecipitated with preimmune serum (p.i.) (lane 5). The positive control for the in vitro translation system, based on comparable E5-ductin interactions observed in vivo (13), was the immunoprecipitation of cotranslated ductin and HAE5 (the E5 fusion protein tagged with the HA1 epitope) with 12CA5 antibody (lane 6) and antiductin antibody (lane 7). Positions of the HAE8 and ductin proteins are indicated. Abs, antibodies.

rabbit reticulocyte lysate (Promega) supplemented with [^{35}S]methionine (Amersham) and amino acid mixture (minus methionine) at a final concentration of 20 mM. Canine pancreas rough microsomal membranes (Promega) were present at a concentration of 3.6 eq per 25 μl of translation mixture. After translation, the mixture was centrifuged at 50,000 rpm for 1 h at 4°C in a Beckman Benchtop ultracentrifuge. The pellet containing the microsomes was solubilized in a sodium dodecyl sulfate (SDS) buffer for electrophoresis or in radioimmunoprecipitation assay (RIPA) buffer for immunoprecipitation.

Transcription products of HAE5 and HAE8 were translated in the reticulocyte system in the absence or presence of microsomes (added either during or after translation). In contrast to ductin, both viral polypeptides were efficiently synthesized in the absence of microsomes (as shown for HAE8 in Fig. 1, lane 3). However, in the presence of microsomes, they partitioned predominantly to the microsomal pellet fraction (as shown for HAE8 in Fig. 1, lane 1). The addition of microsomes to the reaction mixture after translation showed that labelled HAE8 still became associated with the microsomes (data not shown).

The HAE8 polypeptide that partitioned to the microsomal fraction could be present in the lumen of the microsomes, peripherally associated with the microsomal membrane, or directly inserted into the membrane. To distinguish among these three possibilities, an alkaline-treatment experiment was performed. The pellet containing the microsomes with newly synthesized HAE8 was suspended in 50 μl of 100 mM Na_2CO_3 (pH 11), incubated on ice for 1 h, and ultracentrifuged at 50,000 rpm for 1 h at 4°C in a Beckman Benchtop ultracentrifuge to separate integral membrane proteins in the pellet from luminal and peripherally associated proteins in the supernatant (21). HAE8 was found in the pellet (Fig. 1, lane 4), while the secretory protein β-lactamase, used as a control, was found as expected in the supernatant (Fig. 1, lane 7), suggesting that HAE8 is inserted into the lipid bilayer of the microsomal membranes.

Interaction of E5 and E8 with ductin. To examine their interactions with ductin, HAE5 or HAE8 was coexpressed with *Nephrops norvegicus* ductin in the reticulocyte lysate in the presence of microsomes. After centrifugation of the translation reactions, the microsome pellet was solubilized in RIPA buffer for the immunoprecipitation assay. Immunoprecipitations were performed with 1 to 3 μl of the following antibodies incubated overnight at 4°C. Monoclonal antibody 12CA5 raised against the HA1 epitope was obtained from Berkeley Antibody Company (which markets it as 12CA5-H). Antiductin serum (polyclonal N2) was a rabbit polyclonal antiserum made against gap junction isolates from *N. norvegicus* enriched with ductin (17). Unlike with mammalian forms of ductin, antibodies to *Nephrops* ductin are available. *Nephrops* ductin has 82% sequence identity with mammalian forms of ductin overall and 92% identity in the hydrophobic core of the putative transmembrane domains. Protein A-Sepharose was added for 1 h at 4°C. Following three washes in RIPA buffer, Sepharose beads were resuspended in 20 μl of SDS buffer, heated at 100°C for 2 min, and then separated by SDS-polyacrylamide gel electrophoresis (PAGE). Gels were fixed with glacial acetic acid and methanol, dried, and exposed at −70°C.

Figure 2 shows that ductin forms detergent-stable complexes with HAE8 (lanes 2 and 4) and also with HAE5 (lanes 6 and 7).

Specificity of interaction between ductin and E5 or E8. The binding in the cell-free system could be a result of nonspecific hydrophobic interactions between ductin and the viral polypeptides. Therefore, we examined the specificity of bind-

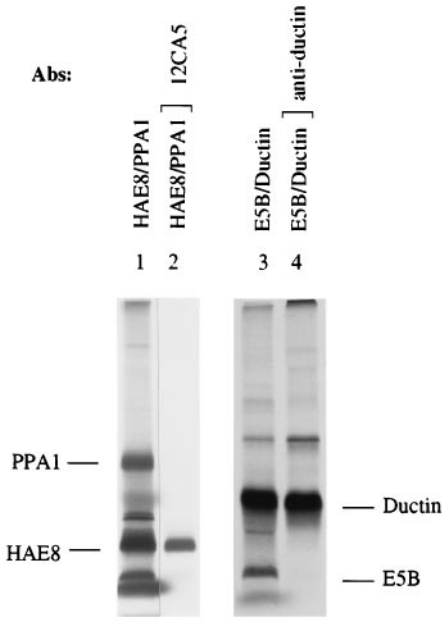

FIG. 3. Specificity of ductin-E8 association. In vitro synthesis in the presence of microsomal membranes of the yeast protein PPA1 with HAE8 is shown in lane 1. Immunoprecipitation with 12CA5 monoclonal antibody, followed by SDS-PAGE (14% polyacrylamide), shows that PPA1 does not bind to HAE8 (lane 2). In vitro synthesis of ductin with the E5B protein is shown in lane 3. Immunoprecipitation with antiductin antibody followed by SDS-PAGE (15% polyacrylamide) shows that E5B does not bind to ductin (lane 4).

ing by substituting related polypeptides for either the viral proteins or ductin.

The PPA1 polypeptide of *Saccharomyces cerevisiae* (1) has a structure homologous to that of ductin, with four hydrophobic domains similarly spaced, and 25% sequence identity. The PPA1 polypeptide, like ductin, partitioned to the microsomal fraction, but after coexpression PPA1 did not coimmunoprecipitate with HAE8 (Fig. 3, lane 2), indicating that it does not interact with the viral oncoprotein.

The 52-amino-acid polypeptide encoded by the E5B ORF of BPV-1 (23) has a structure similar to those of the E5 and E8 polypeptides with a hydrophobic putative transmembrane domain (N-terminal 33 residues) and a hydrophilic C-terminal domain. The E5B ORF of 156 bp was cloned by PCR from an isolate of BPV-1, which diverged from a previously published BPV-1 (5), resulting in five conservative amino acid substitutions (G-4039 to C [G4039C], T4066G, T4093A, T4064C, and T4072G). The E5B polypeptide also partitioned to the microsomal fraction but did not complex with ductin after coexpression (Fig. 3, lane 4). The lack of complex formation between PPA1 and HAE8 and between ductin and E5B shows that the binding between ductin and HAE8 is specific.

Down-regulation of GJIC depends on E8 expression. Ductin is the major component of a connexon channel of gap junctions, and antibodies that bind to ductin block GJIC (10, 27). Likewise, the lipophilic reagent *N*,*N*′-dicyclohexylcarbodiimide, which reacts specifically with a conserved glutamic acid residue in the fourth transmembrane domain of ductin, also blocks GJIC (8, 9). It is therefore possible that the interaction of E8 polypeptide with ductin interferes with GJIC.

To examine this possibility, PalFs, derived from fetal palate as previously described (15), were transfected by a lipofection technique (DOTAP). Following transfection, cells were selected in medium containing G418 (500 μg/ml) for 21 to 28 days. The following plasmids were used. The full BPV-4 genome was cloned in the *Bam*HI site of pAT153 vector (3). The intact E8 and E7 ORFs were cloned in the *Bam*HI site of pSV2neo, under the transcription control of the BPV-4 LCR (15) (E8/E7 in Table 1). The HAE8 ORF was cloned into the *Bam*HI site of pZipneo, and the E7 ORF was cloned into the *Bam*HI site of pZipneo, under the transcription control of the Moloney leukemia virus 5′ long terminal repeat (25) (HAE8 + E7 in Table 1). An activated *ras* gene was carried by the plasmid pT24. Some clones also contained the E6 gene of HPV-16, which confers immortality to the transformed cells and facilitates the isolation of stable transfectants (25). Viral nucleic acids were detected by Southern analysis following established procedures. The expression of BPV-4 E8 and E7 genes was confirmed by reverse transcription-PCR (Perkin Elmer kit) or RNA dot blot hybridization (data not shown).

The level of GJIC was examined by microinjection of lucifer yellow CH into cells as previously described (26). The fluorescent dye diffused from the injected cell to the adjacent cells joined by gap junctions, and the number of fluorescent cells was counted over a 2-minute period (Table 1). Control PalFs showed extensive dye spread (Fig. 4B), while BPV-4-transformed cells showed a marked reduction in GJIC (Fig. 4D). PalFs expressing E8 and E7 showed a similar down-regulation of dye coupling (Fig. 4F) that was not affected by the presence of the HA epitope on E8 (Table 1), whereas PalFs expressing only E7 showed control levels of GJIC (Fig. 4H). HPV-16 E6 had no effect on the level of GJIC (Table 1). These results show that down-regulation of gap junctional communication is dependent on E8 expression.

TABLE 1. Down-regulation of GJIC depends on E8 expression[a]

Viral gene[b]	Clone name	No. of injections	Morphological transformation	GJIC (mean no. of cells coupled ± SD)[c]
PalF (control)		80		26 ± 12
BPV-4	Poly[d]	15	+	5 ± 2
BPV-4	2	15	+	2 ± 2
BPV-4	3	10	+	7 ± 3
BPV-4	4	10	+	8 ± 2
BPV-4	5	10	+	8 ± 4
BPV-4 + 16E6	1	15	+	1 ± 1
BPV-4 + 16E6	2	10	+	4 ± 2
E8/E7 + 16E6	2	15	+	2 ± 2
HAE8 + E7 + 16E6	1	41	+	5 ± 2
HAE8 + E7 + 16E6	5	40	+	6 ± 3
E7	1	15	+	24 ± 5
E7	3	15	+	31 ± 7
E7 + 16E6	1	12	+	22 ± 6
E7 + 16E6	2	15	+	18 ± 10
E7 + 16E6	4	15	+	24 ± 7
E7 + 16E6	5	15	+	20 ± 6
E7 + 16E6	6	20	+	31 ± 10

[a] Cells were microinjected with lucifer yellow, and the total number of cells with detectable fluorescence was counted over a 2-min period. BPV-4-transfected PalFs show a marked down-regulation of GJIC compared with control PalFs. Cells expressing E8 and E7 show a similar marked reduction of dye coupling, whereas cells expressing E7 without E8 show a normal level of GJIC. Cells with or without HPV-16 E6 show similar levels of communication. In all combinations, comparisons using Student's *t* test show that the reduction of GJIC in cultures of E8-containing cells is significant ($P < 0.001$). The data for PalFs were pooled from five different preparations.

[b] All transfectants also contained activated *ras*.

[c] Mean numbers of fluorescent coupled cells ± standard deviations.

[d] Poly indicates a pool of clones.

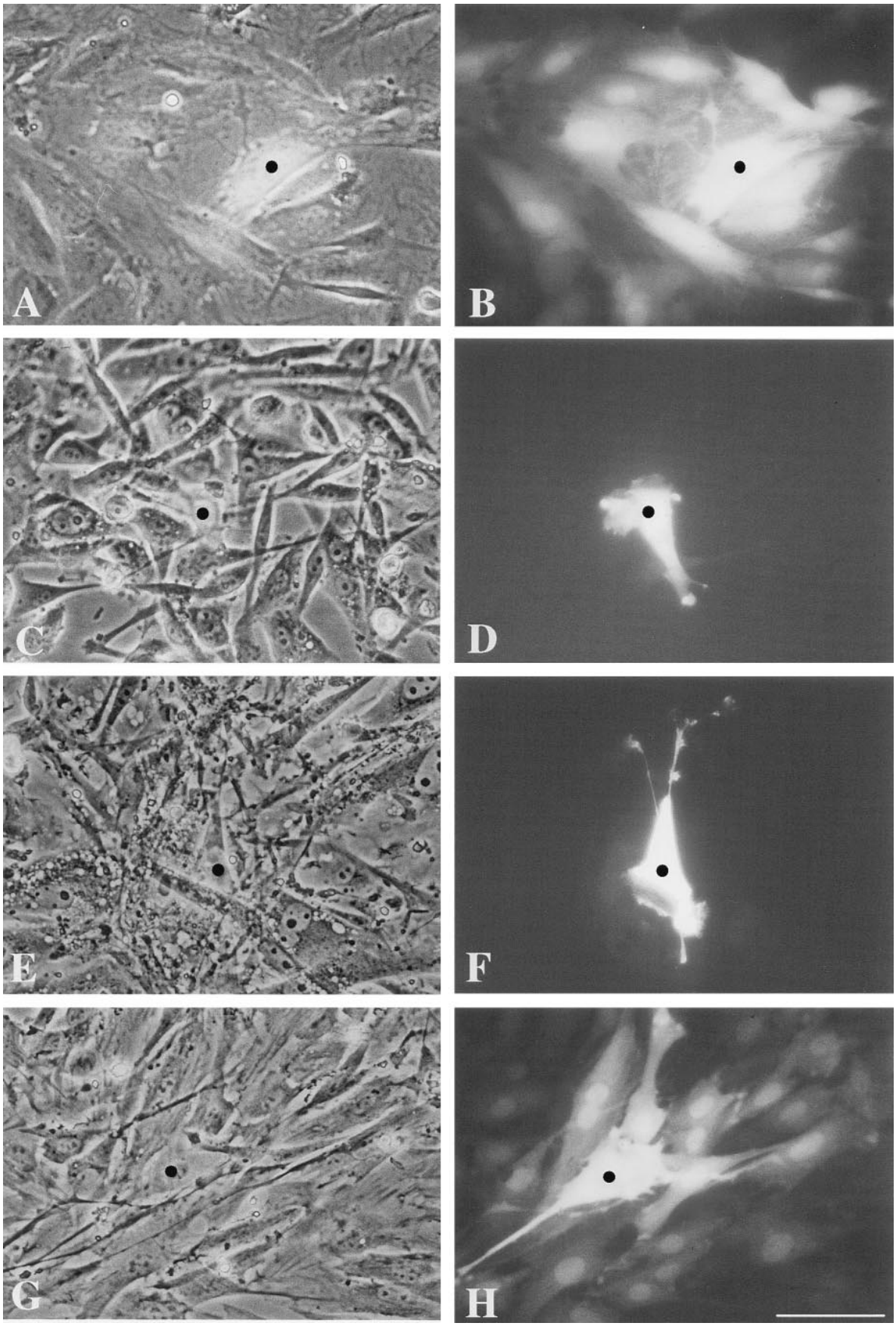
A
B
C
D
E
F
G
H

FIG. 4. Down-regulation of GJIC depends on E8 expression. Normal PalFs (A and B) are joined by functional gap junctions that allow the fluorescent dye lucifer yellow CH to spread from the microinjected cell (marked by a black spot) to surrounding cells. PalFs transfected with BPV-4 (C and D) or E8 and E7 (E and F) are morphologically transformed and show a marked reduction of dye coupling. E7-transfected cells (G and H) are morphologically transformed but show normal levels of dye coupling. Phase-contrast (A, C, E, and G) and fluorescence (B, D, F, and H) micrographs are shown. Scale bar, 25 μm.

Conclusions. In this study, the E8 polypeptide of BPV-4 is shown to interact with ductin. The E8 polypeptide has homology with the BPV-1 E5 polypeptide and with the other small hydrophobic polypeptides which also bind to ductin and are encoded by papillomaviruses (6, 13) and by the retrovirus human T-cell leukemia virus type 1 (12). Therefore, ductin appears to be a common cellular target for this family of virally encoded polypeptides.

Our data showing that E5 and E8 are apparently integral membrane proteins extend previous immunocytochemical analyses that showed that E5 and E8 are membrane-associated polypeptides (2, 25). The association of E8 to the microsomal membranes either during or after synthesis suggests a post-translational insertion of the E8 polypeptide into endoplasmic reticulum membranes in vitro. It is therefore possible that in vivo the E8 polypeptide needs not transit through the endoplasmic reticulum-Golgi network of the cell.

Our study shows a marked loss of GJIC in PalFs transformed by BPV-4 and that this activity depends on the expression of the E8 ORF. Transformation is often associated with a loss of GJIC (20). However, morphological transformation of PalFs by E7 has no significant effect on GJIC. Therefore, cellular transformation alone is not sufficient to cause the loss of GJIC observed in E8-expressing PalFs. As loss of coupling is dependent on the expression of E8, a possible mechanism for the marked reduction in gap junctional communication could be the binding of the E8 polypeptide to ductin, a gap junction structural protein. It is interesting that HPV-16 E5, which previously had been shown to bind to ductin (6), when expressed in the established cell line HaCaT also inhibits GJIC and does so to an extent similar to that found in this study (24).

The down-regulation of GJIC could be important for the release of potential tumor cells from gap junction-mediated growth suppression by surrounding normal cells (20). The finding that E5-related viral oncoproteins can down-regulate GJIC as well as stimulate growth through potentiation of growth factor receptor activity suggests that these proteins have perhaps evolved a strategy of transformation that combines different events.

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REFERENCES

1. **Apperson, M., R. E. Jensen, K. Suda, C. Witte, and M. P. Yaffe.** 1990. A yeast protein, homologous to the proteolipid of the chromaffin granule proton-ATPase, is important for cell growth. Biochem. Biophys. Res. Commun. **168:**574–579.
2. **Burkhardt, A., M. Willingham, C. Gay, K.-T. Jeang, and R. Schlegel.** 1989. The E5 oncoprotein of bovine papillomavirus is oriented asymmetrically in Golgi and plasma membranes. Virology **170:**334–339.
3. **Campo, M. S., and L. W. Coggins.** 1982. Molecular cloning of bovine papillomavirus genomes and comparison of their sequence homologies by heteroduplex mapping. J. Gen. Virol. **63:**255–264.
4. **Campo, M. S., B. W. O'Neil, R. J. Barron, and W. F. H. Jarrett.** 1994. Experimental reproduction of the papilloma-carcinoma complex of the alimentary canal in cattle. Carcinogenesis **15:**1597–1601.
5. **Chen, E. Y., P. M. Howley, A. D. Levinson, and P. H. Seeburg.** 1982. The primary structure and genetic organization of the bovine papillomavirus type I genome. Nature (London) **299:**529–534.
6. **Conrad, M., V. J. Bubb, and R. Schlegel.** 1993. The human papillomavirus type 6 and 16 E5 proteins are membrane-associated proteins which associate with the 16-kilodalton pore-forming protein. J. Virol. **67:**6170–6178.
7. **Dunlop, J., P. C. Jones, and M. E. Finbow.** 1995. Membrane insertion and assembly of ductin: a polytopic channel with dual orientation. EMBO J. **14:**3609–3616.
8. **Finbow, M. E., E. E. Eliopoulos, P. J. Jackson, J. N. Keen, L. Meagher, P. Thompson, P. Jones, and J. B. C. Findlay.** 1992. Structure of a 16 kDa integral membrane protein that has identity to the putative proton channel of the vacuolar H^+-ATPase. Protein Eng. **5:**7–15.
9. **Finbow, M. E., S. John, E. Kam, D. K. Apps, and J. D. Pitts.** 1993. Disposition and orientation of ductin (DCCD-reactive vacuolar H^+-ATPase subunit) in mammalian membrane complexes. Exp. Cell Res. **207:**261–270.
10. **Finbow, M. E., and J. D. Pitts.** 1993. Is the gap junction channel—the connexon—made of connexin or ductin? J. Cell Sci. **106:**463–472.
11. **Finbow, M. E., J. D. Pitts, D. J. Goldstein, R. Schlegel, and J. B. C. Findlay.** 1991. The E5 oncoprotein target: a 16-kDa channel-forming protein with diverse functions. Mol. Carcinog. **4:**441–444.
12. **Franchini, G., J. C. Mulloy, I. J. Koralnik, A. Lo Monico, J. J. Sparkowski, T. Andresson, D. J. Goldstein, and R. Schlegel.** 1993. The human T-cell leukemia/lymphotropic virus type I $p12^I$ protein cooperates with the E5 oncoprotein of bovine papillomavirus in cell transformation and binds the 16-kilodalton subunit of the vacuolar H^+ ATPase. J. Virol. **67:**7701–7704.
13. **Goldstein, D. J., M. E. Finbow, T. Andresson, P. McLean, K. T. Smith, V. Bubb, and R. Schelegel.** 1991. Bovine papillomavirus E5 binds to the 16k component of vacuolar H^+-ATPases. Nature (London) **352:**347–349.
14. **Goldstein, D. J., and R. Schlegel.** 1990. The E5 oncoprotein of bovine papillomavirus binds to a 16 kd cellular protein. EMBO J. **9:**137–146.
15. **Jaggar, R. T., W. D. Pennie, K. T. Smith, M. E. Jackson, and M. S. Campo.** 1990. Co-operation between bovine papillomavirus type 4 and *ras* in the morphological transformation of primary bovine fibroblasts. J. Gen. Virol. **71:**3041–3046.
16. **Jarrett, W. F. H., P. E. McNeil, W. T. R. Grimshaw, I. E. Selman, and W. I. M. McIntyre.** 1978. High incidence area of cattle cancer with a possible interaction between an environmental carcinogen and a papilloma virus. Nature (London) **274:**215–217.
17. **Leitch, B., and M. E. Finbow.** 1990. The gap junction-like form of a vacuolar proton channel appears not to be an artefact of isolation: an immunocytochemical localisation study. Exp. Cell Res. **190:**218–226.
18. **Mandel, M., Y. Moriyama, J. D. Hulmes, Y. C. Pan, H. Nelson, and N. Nelson.** 1988. cDNA sequence encoding the 16 kDa proteolipid of chromaffin granules implies gene duplication in the evolution of H^+-ATPase. Proc. Natl. Acad. Sci. USA **85:**5521–5524.
19. **Martin, P., W. Vass, J. T. Schiller, D. Lowry, and T. J. Velu.** 1989. The bovine papillomavirus E5 transforming protein can stimulate the transforming activity of EGF and CSF-1 receptors. Cell **59:**21–32.
20. **Mesnil, M., and H. Yamasaki.** 1993. Cell-cell communication and growth control of normal and cancer cells: evidence and hypothesis. Mol. Carcinog. **7:**14–17.
21. **Miao, G.-H., Z. Hong, and D. P. S. Verma.** 1992. Topology and phosphorylation of soybean nodulin-26, an intrinsic protein of the peribacteroid membrane. J. Cell Biol. **118:**481–490.
22. **Nelson, N.** 1992. Organellar proton-ATPase. Curr. Opin. Cell Biol. **4:**654–660.
23. **O'Banion, M. K., V. D. Winn, J. Settleman, and D. A. Young.** 1993. Genetic definition of a new bovine papillomavirus type 1 open reading frame, E5B, that encodes a hydrophobic protein involved in altering host-cell protein processing. J. Virol. **67:**3427–3434.
24. **Oelze, I., J. Kartenbeck, K. Crusius, and A. Alonso.** 1995. Human papillomavirus type 16 E5 protein affects cell-cell communication in an epithelial cell line. J. Virol. **69:**4489–4494.
25. **Pennie, W. D., G. J. Grindlay, M. Cairney, and M. S. Campo.** 1993. Analysis of the transforming functions of bovine papillomavirus type 4. Virology **193:**614–620.
26. **Pitts, J. D., and E. Kam.** 1985. Communication compartments in mixed cell cultures. Exp. Cell Res. **156:**439–449.
27. **Serras, F., T. E. J. Buultjens, and M. E. Finbow.** 1988. Inhibition of dye coupling in *Patella* (Mollusca) by microinjecton of antiserum against *Nephrops* (Arthropoda) gap junctions. Exp. Cell Res. **179:**282–288.
28. **Straight, S. W., B. Herman, and D. J. McCance.** 1995. The E5 oncoprotein of human papillomavirus type 16 inhibits the acidification of endosomes in human keratinocytes. J. Virol. **69:**3185–3192.
29. **Straight, S. W., P. M. Hinkle, R. J. Jewers, and D. J. McCance.** 1993. The E5 oncoprotein of human papillomavirus type 16 transforms fibroblasts and effects the downregulation of the epidermal growth factor receptor in keratinocytes. J. Virol. **67:**4521–4532.
30. **Waters, C. M., K. A. Overholser, A. Sorkin, and G. Carpenter.** 1992. Analysis of the influence of E5 transforming protein on kinetic parameters of epidermal growth factor binding and metabolism. J. Cell. Physiol. **152:**253–263.