Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4634–4638, May 1994
Biochemistry

Cellular transformation by a transmembrane peptide: Structural requirements for the bovine papillomavirus E5 oncoprotein

APRIL N. MEYER, YOU-FENG XU, MELANIE K. WEBSTER, AMELIA E. SMITH, AND DANIEL J. DONOGHUE*

Department of Chemistry/Division of Biochemistry and Center for Molecular Genetics, University of California at San Diego, La Jolla, CA 92093–0322

Communicated by Bruno H. Zimm, February 7, 1994

ABSTRACT **The E5 oncoprotein of bovine papillomavirus, only 44 amino acids long, occurs as a disulfide-bonded transmembrane dimer. This remarkable oncoprotein stimulates signal transduction through activation of the platelet-derived growth factor (PDGF) receptor, and E5 exhibits limited amino acid sequence similarity with PDGF. Results presented here suggest that a key feature of the hydrophobic transmembrane domain is an amino acid side chain that participates in interhelical hydrogen bond formation. These data are reminiscent of the activated *neu* oncogene, in which a point mutation in the transmembrane domain leads to ligand-independent dimerization and activation of a receptor tyrosine kinase. Significantly, the transmembrane domain of E5 can be largely replaced by the transmembrane domain from the activated *neu* receptor tyrosine kinase. Extensive mutagenesis defines the minimal structural features required for transformation by the E5 oncoprotein as, first, the ability to dimerize and, second, presentation of a negatively charged residue at the extracellular side of the membrane. The biological activity of E5 mutants that lack most amino acid residues similar to PDGF suggests that E5 and PDGF activate the PDGF receptor by distinct mechanisms.**

Bovine papillomavirus type I (BPV) typifies the fibropapilloma viruses, which transform fibroblasts *in vitro* and lead to development of fibrosarcomas in their host. In contrast, the human papillomaviruses infect epithelial cells and are strongly implicated in carcinomas of the cervix and respiratory tract (1). The fibroblast-transforming function of BPV is due to a small open reading frame, designated E5, encoding a 44-residue protein that forms membrane-associated disulfide-bonded dimers (2–6). Recent work has demonstrated that the E5 oncoprotein activates the platelet-derived growth factor (PDGF) β-receptor (7, 8) and may display functional similarity with the human T-lymphotropic virus type I $p12^I$ protein (9).

Inspection of the amino acid sequence of the E5 oncoprotein reveals two distinct domains (2–6): an extremely hydrophobic N-terminal domain, residues 1–32, and a hydrophilic C-terminal domain, residues 33–44. Several studies have led to the conclusion that the E5 oncoprotein is membrane-anchored with a type II orientation, with the N terminus intracellular and the C terminus extracellular (10). In addition, the hydrophobic domain of E5 can function as a signal-anchor domain, indistinguishable from signal-anchor domains of well-characterized type II proteins such as neuraminidase, transferrin receptor, or asialoglycoprotein receptor (11).

In this work, we describe the minimal structural features required for biological transforming activity by a transmembrane peptide. The results presented here suggest that E5 and PDGF activate the PDGF receptor by fundamentally distinct mechanisms.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Construction of Mutants. Mutants were derived by *in vitro* synthesis of two complementary oligonucleotides that, when hybridized, create a small restriction fragment with *Xho* I and *Cla* I cohesive termini. This fragment was directly ligated into a Moloney murine leukemia virus-derived retroviral vector (12). Optimized oligonucleotide synthesis and purification were as described (11). Recovered recombinant clones were confirmed by nucleotide sequencing of the entire insert. Where designated, only the C-terminal half of the E5 gene was resynthesized with *Nco* I and *Cla* I overhangs. This was inserted into a vector carrying the silent E5 mutation Q17E, which allows creation of a unique *Nco* I site at codons 15–17 of the E5 gene, with the sequence . . .G*CC ATG* GAA. . . coding for Ala-15 Met-16 Glu-17. Comparison of the Q17E mutant with the native E5 oncogene indicated no detectable difference in the number or size of foci in transformation assays.

E5/*neu* Substitution Mutant. Degenerate oligonucleotides were synthesized that would encode a pool of E5/*neu* transmembrane recombinants in which the 44 residues would be derived as follows: 1–5 from E5, 6–10 from E5 or *neu*, 11–17 from *neu*, 18–30 from E5 or *neu*, and 31–44 from E5. From an initial pool of mutants, many individual isolates were recovered by PCR techniques and sequenced, including the biologically active E5/*neu* recombinant shown in Fig. 1.

Focus Assays. For focus assays, NIH 3T3 cells were transfected as described (12). Foci were scored after 5 days. All mutants were assayed at least twice in independent experiments.

Epitope-Tagged Derivatives and Immunoprecipitations. Epitope-tagged derivatives were constructed by replacement of the native C-terminal residues TGLPF (residues 40–44) with the epitope-tag HSLPFML (as residues 40–46), which has been described (13). Acutely infected NIH 3T3 cells expressing either the E5-epi construct, with native disulfide bonds, or the C37A S38C C39A-epi mutant were labeled for 4 hr with ≈200 μCi (1 Ci = 37 GBq) each of [^{35}S]Cys and [^{35}S]Met per ml in minimum essential medium lacking Cys and Met. Immunoprecipitation (12) was carried out using affinity-purified antibody 417, which specifically recognizes the epitope-tag (13), and immune complexes were collected with protein A-Sepharose. After immune complex formation, samples were boiled in sample buffer in the absence or presence of 2-mercaptoethanol to reduce disulfide-bonded molecules. Samples were separated on a 17.5% SDS/polyacrylamide gel and processed for fluorography to visualize proteins.

RESULTS AND DISCUSSION

Requirements of the Transmembrane Domain. Given that the E5 hydrophobic domain can function as a signal-anchor

Abbreviations: BPV, bovine papillomavirus type I; PDGF, platelet-derived growth factor.

*To whom reprint requests should be addressed.

domain (11), we initially assumed that heterologous signal-anchor domains would functionally substitute for the E5 hydrophobic domain. E5 derivatives were constructed with the hydrophobic domain substituted by transmembrane domains of various type II proteins, including neuraminidase (14) and three different glycosyltransferases (15–17). Surprisingly, none of these constructs exhibited any activity in transformation assays (data not shown). This suggested that the transmembrane domain of the E5 oncoprotein serves one of either two specific functions. First, a specialized transmembrane domain might promote association of E5 with another protein required for its activity. Second, a specialized transmembrane domain might exhibit specific structural features required for packing the two subunits into the dimeric molecule. These specific structural features would not be intrinsic to the transmembrane domains of type II proteins such as neuraminidase or glycosyltransferases.

Examination of the transmembrane domain of the E5 oncoprotein reveals no charged residues and few polar residues, the notable exception being Gln-17. Other investigators have suggested that Gln-17 is required for association and coimmunoprecipitation of E5 with the 16-kDa subunit of vacuolar ATPase, and mutation of Gln-17 to either Gly or Leu correlates with loss of 16-kDa association (18–20). Such studies imply that the side chain of residue Gln-17 must lie outside of the helical "interface" of the transmembrane dimer to allow hydrogen bonding with other membrane proteins. However, energetic considerations of helical dimer formation in the membrane environment suggest that dimerization may be "driven" by the requirement to bury polar residues, such as Gln-17, within the interfacial region of the helical dimer (21, 22).

One example of transmembrane dimerization is provided by the *neu* oncoprotein, a receptor tyrosine kinase that exhibits the opposite membrane orientation (type I) and no amino acid similarity to the E5 oncoprotein. Mutation of Val-664 to Glu, within the transmembrane domain, leads to ligand-independent dimerization of the *neu* receptor tyrosine kinase with concomitant receptor activation and signal transduction (23). Mutagenesis studies have confirmed the structural importance of the pentapeptide subdomain extending from residue 661 to 665, including the key residue Glu-664, and modeling studies have suggested interhelical hydrogen bonding between the side chain of Glu-664 and the carbonyl oxygen of Ala-661 of the other helix (24–26). Various point mutations at Val-664 of *neu* have been examined and exhibit the following pattern of activity with respect to transformation assays: E = Q > D, Y >> V, K, G, H (27).

Twelve point mutants were constructed to further define the role of Gln-17 of the E5 oncoprotein (Table 1). Interestingly, the mutants Q17E and Q17K exhibited wild-type transformation activity and the mutant Q17H exhibited decreased but significant activity. These substitutions can be summarized as follows: Q = E = K > H >> R, D, N, Y, L, M, V, C, G. These results suggest that the essential feature at this position is an amino acid side chain capable of hydrogen bond formation such as Gln, Glu, or Lys but that hydrogen bond donors/acceptors with shorter side chains such as Asp and Asn cannot substitute. The low activity of the Q17H mutant can be explained by the ability of His to participate in hydrogen bond formation with a bond length intermediate between Gln and Asn (28).

We are currently using molecular modeling to examine two different models for interhelical hydrogen bonding. In one model, the side chain of residue 17 of each subunit would act as the hydrogen bond donor, forming a hydrogen bond to the α-helical backbone of the opposite subunit, with the carbonyl oxygen of residue 14 as the hydrogen bond acceptor. In the second model, the side chain of residue 17 of one subunit would form a hydrogen bond with the side chain of residue 17 of the opposite subunit—e.g., for the Glu-17 mutant, the δ-carboxylate anion of one side chain would act as the hydrogen bond acceptor or base, and the δ-carboxylic acid of the other side chain would act as the hydrogen bond donor.

Table 1. Transformation efficiencies of E5 transmembrane mutants.

Mutant	Transformation
E5 wild type	+++
Q17E	+++
Q17K	+++
Q17H	+*
Q17R	−
Q17D	−
Q17N	−
Q17Y	−
Q17L	−
Q17M	−
Q17V	−
Q17C	−
Q17G	−
E5/*neu* substitution	++*

Transformation efficiencies of E5 mutants are defined in legend in Fig. 3. Sequence of the E5/*neu* substitution mutant is shown in Fig. 1. *Foci of smaller size than E5 wild type.

The Hydrophobic Domain of E5 Can Be Largely Exchanged with the *neu* Transmembrane Domain. We attempted to swap the transmembrane domain of *neu* in place of that of the E5 oncoprotein. Eight different substitution mutants were constructed, containing as few as 8 residues or as many as 23 residues from *neu* substituted into the transmembrane domain of E5 (data not shown). All of these initial substitutions resulted in biologically inactive proteins, suggesting strict structural constraints for a functional transmembrane domain.

We therefore devised a selection protocol that would identify E5/*neu* substitution mutants with allowable amino acid sequences. Degenerate oligonucleotides were designed to encode E5 derivatives substituted with a minimum of seven residues from *neu* and in which the flanking residues could be derived *either* from *neu* or E5. Fig. 1 shows one transforming isolate that clearly possesses the pentapeptide "core" region of *neu* (24–26), including the Glu-664 residue. This isolate exhibits 18/25 identical residues when compared with the *neu* oncoprotein, and the remainder represent mostly conservative substitutions. The difficulty in recovering active E5/*neu* substitution mutants, however, emphasizes the importance of structural details of individual side chains as they pack into an α-helix. (Schematic drawings of the native E5 oncoprotein and the E5/*neu* substitution mutant described here are presented in Fig. 5*A* and *B*, respectively.) These results clearly demonstrate that the transmembrane domain of E5 can be substituted by a heterologous

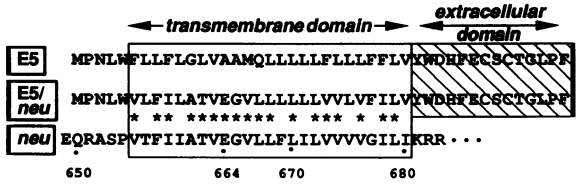

FIG. 1. Sequence of biologically active E5/*neu* recombinant oncoprotein. The transmembrane domain of the E5/*neu* substitution mutant is largely derived from the transmembrane domain of the activated *neu* receptor tyrosine kinase. The putative boundaries of the transmembrane domain and the extracellular domain are shown. Identities within the transmembrane domains between E5/*neu* and the activated *neu* oncoprotein are indicated by asterisks.

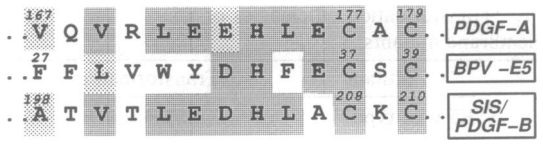

FIG. 2. Alignments of E5 with PDGF-A and PDGF-B. Amino acid similarities between the extracellular domain of BPV-E5 and the C-terminal regions of the transforming domains of PDGF-A and *SIS*/PDGF-B are shown. Heavy stippling indicates identities, whereas light stippling indicates similarities.

transmembrane domain, even one normally inserted in the opposite orientation such as *neu*.

Scanning Mutagenesis of the Extracellular Domain. Prior studies have suggested that activation of the PDGF β-receptor by the E5 oncoprotein may be due to amino acid sequence similarity between PDGF and the extracellular domain of E5 (7) (Fig. 2). Therefore, each residue in the extracellular domain from Phe-28 through Glu-36 was individually mutated to Ala. Surprisingly, all of the alanine scanning mutants from F28A through E36A were active (Fig. 3*A*, mutants 1–9), indicating that no single residue is indispensable for activity.

We considered the possibility that two specific amino acids from among the extracellular residues Asp-33, His-34, Phe-35, or Glu-36 might function redundantly in receptor activation, and we therefore constructed all possible double mutants involving these residues (Fig. 3*B*). Of particular interest was the possibility that Asp-33 and Glu-36 may provide for redundancy with respect to a negatively charged side chain. Consistent with this hypothesis, the double mutant D33A E36A (mutant 12) was devoid of biological activity. Moreover, the double mutant H34A F35A (mutant 13), which leaves Asp-33 and Glu-36 intact, retained biological activity demonstrating that a negative charge is the *only* significant requirement in this region. Thus, provided that the disulfide bonds are maintained, the only specific requirement in the extracellular domain appears to be either the acidic residue Asp-33 or Glu-36.

Further analysis of double mutants suggests a more profound role for Asp-33 than for Glu-36 (Fig. 3*B*). When the D33A mutation was assayed as the double mutant D33A H34A (mutant 10) or as the double mutant D33A F35A (mutant 11), activity was lost, suggesting some contribution from His-34 or Phe-35. However, biological activity was retained when the E36A mutation was similarly assayed in combination with mutations at His-34 or Phe-35 (mutants 14 and 15).

E5 Mutants with Novel Disulfide Bonds. The possibility that the disulfide bonds are arranged in a trans configuration—i.e., Cys-37 of one joined to Cys-39 of the other subunit—suggested that the extracellular domains of the two subunits may be juxtaposed in an antiparallel configuration. Fig. 3*C* presents cysteine scanning mutagenesis in which a pair of symmetry-related residues was mutated to Cys, in an effort to compensate for the loss of the native Cys residues. Two mutants displayed significant biological activity: the H34C C37A C39A L42C mutant (mutant 19) and the E36C C37A C39A T40C mutant (mutant 21). This demonstrates that the native disulfide bonds can be substituted by novel disulfide bonds in the extracellular domain.

A

27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44	ACTIVITY/MUTANT #
F F L V Y W D H F E C S C T G L P F	
A	+++ #1
A	+++ #2
A	+++ #3
A	+++ #4
A	+++ #5
A	+++ (b) #6
A	+++ (b) #7
A	+++ (b) #8
A	+++ (b) #9

B

27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44		
F F L V Y W D H F E C S C T G L P F		
A A	− (b)	#10
A A	− (b)	#11
A A	− (b)	#12
A A	+++ (b)	#13
A A	+++ (b)	#14
A A	+++ (b)	#15

C

27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44		
F F L V Y W D H F E C S C T G L P F		
A A	−	#16
C A A C	−	#17
C A A C	−	#18
C A A C	+++ (a)	#19
C A A C	−	#20
C A A C	++	#21
C C	+++	wild type
A C A	++ (a)	#22

D

27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44		
F F L V Y W D H F E C S C T G L P F		
C- COOH	+++ (b)	#23
C- COOH	+++ (b)	#24
C- COOH	+++ (a,b)	#25
C- COOH	− (b)	#26
C- COOH	− (b)	#27

FIG. 3. Mutants in the extracellular domain of the E5 oncoprotein. (*A*) "Alanine scanning" mutants. (*B*) Double alanine point mutants. (*C*) "Cysteine scanning" mutants. (*D*) Single cysteine deletion mutants. Mutation to Cys is indicated by C and mutation to Ala is indicated by A. Efficiencies of transformation relative to E5 wild type are represented as follows: +++, 20–100%; ++, 5–20%; +, 1–5%; and −, <1%. (a), Foci of smaller size than E5 wild type; (b), the mutant also carries the Q17E mutation (see text).

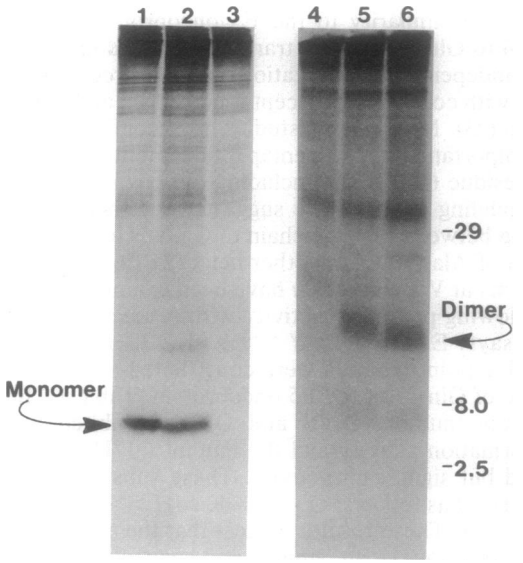

FIG. 4. SDS/PAGE of immunoprecipitated E5 derivative, C37A S38C C39A-epi. Due to the presence of only a single Cys residue, recovery of a dimeric molecule indicates dimerization through the single intermolecular disulfide bond involving Cys-38 of each subunit. This mutant was analyzed in comparison with an epitope-tagged derivative of wild-type E5, designated E5-epi, which retains the native Cys residues, Cys-37 and Cys-39. Molecular mass markers are shown in kDa. Exposure time was 7 days. Lanes 1–3, immunoprecipitated proteins analyzed by SDS/PAGE under *reducing* conditions, in the presence of 2-mercaptoethanol. Lane 1, E5-epi; lane 2, C37A S38C C39A-epi; lane 3, mock-transfected NIH 3T3 cells as negative control. Lanes 4–6, Immunoprecipitated proteins analyzed by SDS/PAGE under *nonreducing* conditions. Lane 4, mock-transfected NIH 3T3 cells as negative control; lane 5, E5-epi; lane 6, C37A S38C C39A-epi.

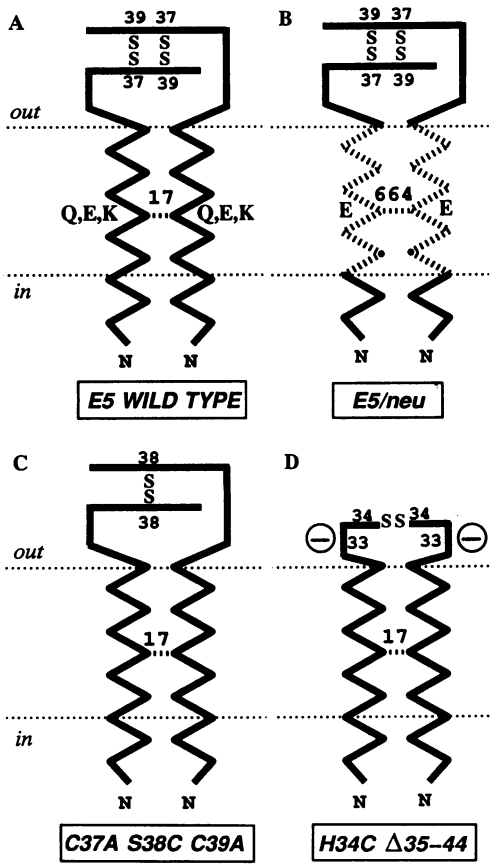

FIG. 5. Schematic drawings of E5 dimer. (*A*) E5 is shown as a type II transmembrane protein with parallel α-helices. The extracellular domain is shown with the native disulfide bonds in an antiparallel arrangement. "N" refers to the amino terminus of the E5 oncoprotein, and "in" and "out" refer to the intracellular and extracellular sides of the membrane. Disulfide bonds are represented as "S S." Hydrogen bonding involving residue 17 is shown, which can be substituted by either Gln, Glu, or Lys ("Q,E,K"). (*B*) Diagrammatic representation of the E5/*neu* recombinant, with the transmembrane domain largely substituted by the transmembrane domain of the activated *neu* oncoprotein, shown by the stippled line. Hydrogen bonding involving residue Glu-664 of the activated *neu* oncoprotein is indicated ("E664"). The sequence of this mutant is shown in Fig. 1. (*C*) Diagrammatic representation of the E5 mutant C37A S38C C39A (Fig. 3*C*, mutant 22), with a single disulfide bond involving residue 38 of each subunit. The single disulfide bond replaces the two native disulfide bonds. (*D*) Diagrammatic representation of the minimal E5 mutant H34C Δ35–44 (Fig. 3*C*, mutant 25), in which most of the extracellular domain has been deleted. Compared with the native extracellular domain, only a single negatively charged residue remains, Asp-33, and a single Cys residue has been introduced at residue 34 to provide for disulfide bonding. The negative charge indicates the side chain of Asp-33.

The results above also suggested that a single disulfide bond located at residue 38 of each subunit might be sufficient for transforming activity, as demonstrated by the triple mutant C37A S38C C39A (Fig. 3*C*, mutant 22). Disulfide bond formation between the novel residue Cys-38 of each subunit was demonstrated using an epitope-tagged derivative, which was recovered by immunoprecipitation (Fig. 4). This experiment yielded a dimeric protein when analyzed under nonreducing conditions. Fig. 5*C* shows a diagrammatic representation of the C37A S38C C39A mutant (mutant 22), with only a single disulfide bond, in comparison with wild-type E5 (Fig. 5*A*).

To further examine the requirement for a Cys residue, we systematically increased the extent of the C-terminal deletion while mutating the last C-terminal residue of each mutant to Cys. Fig. 3*D* reveals that a transforming phenotype *required only the minimal retention of Asp-33*, together with a Cys residue for disulfide bonding, as demonstrated by the H34C Δ35–44 mutant (mutant 25), which retains Asp-33 (see diagrammatic representation in Fig. 5*D*). Thus, the E5 oncoprotein displays remarkable flexibility in the location of its disulfide bond(s), more so than any other growth factor.

Relationship of E5 Oncoprotein to PDGF. The identification of specific residues in PDGF that are responsible for receptor activation has been intensively investigated by several laboratories (29–33). The ability of the E5 oncoprotein to activate the PDGF β-receptor, together with its slight amino acid similarity with PDGF, suggests that the E5 oncoprotein may function as a miniature analog of PDGF. However, examination of the recently described 3.0-Å-resolution structure of PDGF BB (34) shows little or no structural similarity to E5, and the connectivity of the Cys residues is different in E5 compared with PDGF.

We favor a model for PDGF receptor activation by the E5 oncoprotein in which one or more residues of the E5 extracellular domain may bind to the juxtamembrane region of the PDGF receptor, thereby inducing receptor dimerization and activation. This binding could be mediated by the negatively charged residue(s) Asp-33 or Glu-36, present in the extracellular domain of the E5 oncoprotein, interacting with the positively charged residue Lys-499 of the PDGF β-receptor (35). This model is consistent with the biological activity of E5 mutants in which the extracellular domain has been largely deleted, including most of those residues exhibiting similarity to PDGF (Fig. 2). This model suggests that receptor activation by E5 is fundamentally different than for PDGF, which clearly interacts with the large ligand binding domain of the PDGF receptor (36).

Some eukaryotic viruses encode highly specific growth factors that mitogenically stimulate infected cells and thereby assist viral replication, such as vaccinia virus growth factor, which structurally resembles epidermal growth factor (EGF) and activates the EGF receptor (37). In contrast, the E5 oncoprotein bears little structural resemblance to the growth factor that it mimics. The data presented here demonstrate that only minimal structural features are required for growth factor receptor activation by viral-encoded transmembrane peptides. Moreover, this suggests that other viruses may encode unidentified mitogenic peptides as small and structurally unrecognizable as the papillomavirus E5 oncoprotein.

We thank Laura J. Castrejon for administrative support, Lucinda Chen for technical assistance, and Kyle Durrick, Kristen Hart, and Daniel Van Antwerp for enthusiastic assistance during laboratory rotations. In addition, we thank Richard Blewitt, Jon Singer, Russell Doolittle, Charles Perrin, David Roise, Maurice Montal, and Joe Kraut for valuable suggestions. This work was supported by Grant CA 40573 from the National Institutes of Health, by an American Cancer Society Faculty Research Award, and by the Lucille P. Markey Charitable Trust.

1. Pfister, H., ed. (1990) *Papillomaviruses and Human Cancer* (CRC, Boca Raton, FL).
2. DiMaio, D., Guralski, D. & Schiller, J. T. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1797–1801.
3. Horwitz, B. H., Burkhardt, A. L., Schlegel, R. & DiMaio, D. (1988) *Mol. Cell. Biol.* **8**, 4071–4078.
4. Settleman, J., Fazeli, A., Malicki, J., Horwitz, B. H. & DiMaio, D. (1989) *Mol. Cell. Biol.* **9**, 5563–5572.
5. Schlegel, R., Wade-Glass, M., Rabson, M. S. & Yang, Y.-C. (1986) *Science* **233**, 464–467.
6. Schiller, J. R., Vass, W. C., Vousden, K. H. & Lowy, D. R. (1986) *J. Virol.* **57**, 1–6.
7. Petti, L., Nilson, L. A. & DiMaio, D. (1991) *EMBO J.* **10**, 845–855.
8. Petti, L. & DiMaio, D. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6736–6740.

9. Koralnik, I. J., Fullen, J. & Franchini, G. (1993) *J. Virol.* **67,** 2360–2366.
10. Burkhardt, A., Willingham, M., Gay, C., Jeang, K.-T. & Schlegel, R. (1989) *Virology* **170,** 334–339.
11. Xu, Y.-F., Meyer, A. N., Webster, M. K., Lee, B. A. & Donoghue, D. J. (1993) *J. Cell Biol.* **123,** 549–560.
12. Maher, D. W., Strawn, L. M. & Donoghue, D. J. (1993) *Oncogene* **8,** 533–541.
13. Pickham, K. M., Meyer, A. N., Li, J. & Donoghue, D. J. (1992) *Mol. Cell. Biol.* **12,** 3192–3203.
14. Brown, D. J., Hogue, B. G. & Nayak, D. P. (1988) *J. Virol.* **62,** 3824–3831.
15. Munro, S. (1991) *EMBO J.* **10,** 3577–3588.
16. Joziasse, D. H., Shaper, J. H., Van den Eijnden, D. H., Van Tunen, A. J. & Shaper, N. L. (1989) *J. Biol. Chem.* **264,** 14290–14297.
17. Machamer, C. E. (1991) *Trends Cell Biol.* **1,** 141–144.
18. Goldstein, D. J., Finbow, M. E., Andresson, T., McLean, P., Smith, K., Bubb, V. & Schlegel, R. (1991) *Nature (London)* **352,** 347–349.
19. Goldstein, D. J., Kulke, R., DiMaio, D. & Schlegel, R. (1992) *J. Virol.* **66,** 405–413.
20. Kulke, R., Horwitz, B. H., Zibello, T. & DiMaio, D. (1992) *J. Virol.* **66,** 505–511.
21. Popot, J.-L. & Engelman, D. M. (1990) *Biochemistry* **29,** 4031–4037.
22. Singer, S. J. (1990) *Annu. Rev. Cell Biol.* **6,** 247–296.
23. Bargmann, C. I., Hung, M.-C. & Weinberg, R. A. (1986) *Nature (London)* **319,** 226–230.
24. Gullick, W. J., Bottomley, A. C., Lofts, F. J., Doak, D. G., Mulvey, D., Newman, R., Crumpton, M. J., Sternberg, M. J. E. & Campbell, I. D. (1992) *EMBO J.* **11,** 43–48.
25. Sternberg, M. J. E. & Gullick, W. J. (1989) *Nature (London)* **339,** 587.
26. Cao, H., Bangalore, L., Bormann, B. J. & Stern, D. F. (1992) *EMBO J.* **11,** 923–932.
27. Bargmann, C. I. & Weinberg, R. A. (1988) *EMBO J.* **7,** 2043–2052.
28. Lowe, D. M., Fersht, A. R. & Wilkinson, A. J. (1985) *Biochemistry* **24,** 5106–5109.
29. Clements, J. M., Bawden, L. J., Bloxidge, R. E., Catlin, G., Cook, A. L., Craig, S., Drummond, A. H., Edwards, R. M., Fallon, A., Green, D. R., Hellewell, P. G., Kirwin, P. M., Nayee, P. D., Richardson, S. J., Brown, D., Chahwala, S. B., Snarey, M. & Winslow, D. (1991) *EMBO J.* **10,** 4113–4120.
30. Ostman, A., Andersson, M., Hellman, U. & Heldin, C.-H. (1991) *J. Biol. Chem.* **266,** 10073–10077.
31. Giese, N., LaRochelle, W. J., May-Siroff, M., Robbins, K. C. & Aaronson, S. A. (1990) *Mol. Cell. Biol.* **10,** 5496–5501.
32. Engstrom, U., Engstrom, A., Ernlund, A., Westermark, B. & Heldin, C. H. (1992) *J. Biol. Chem.* **267,** 16581–16587.
33. LaRochelle, W. J., Pierce, J. H., May-Siroff, M., Giese, N. & Aaronson, S. A. (1992) *J. Biol. Chem.* **267,** 17074–17077.
34. Oefner, C., D'Arcy, A., Winkler, F. K., Eggimann, B. & Hosang, M. (1992) *EMBO J.* **11,** 3921–3926.
35. Yarden, Y., Escobedo, J. A., Kuang, W.-J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ando, M. E., Harkins, R. N., Francke, U., Fried, V. A., Ullrich, A. & Williams, L. T. (1986) *Nature (London)* **323,** 226–232.
36. Williams, L. T. (1989) *Science* **243,** 1564–1570.
37. Stroobant, P., Rice, A. P., Gullick, W. J., Cheng, D. J., Kerr, I. M. & Waterfield, M. D. (1985) *Cell* **42,** 383–393.