Functional Analysis of Desmoplakin Domains: Specification of the Interaction with Keratin Versus Vimentin Intermediate Filament Networks

Thaddeus S. Stappenbeck, Elayne A. Bornslaeger, Connie M. Corcoran, Hue H. Luu, Maria Luisa A. Virata and Kathleen J. Green

Departments of Pathology and Dermatology, and the Robert H. Lurie Cancer Center, Northwestern University Medical School, Chicago, Illinois 60611

Abstract. We previously demonstrated that truncated desmoplakin I (DP I) molecules containing the carboxyl terminus specifically coalign with and disrupt both keratin and vimentin intermediate filament (IF) networks when overexpressed in tissue culture cells (Stappenbeck, T. S., and K. J. Green. *J. Cell Biol.* 116:1197–1209). These experiments suggested that the DP carboxyl-terminal domain is involved either directly or indirectly in linking IF with the desmosome. Using a similar approach, we have now investigated the behavior of ectopically expressed full-length DP I in cultured cells. In addition, we have further dissected the functional sequences in the carboxyl terminus of DP I that facilitate the interaction with IF networks. Transient transfection of a clone encoding full-length DP I into COS-7 cells produced protein that appeared in some cells to associate with desmosomes and in others to coalign with and disrupt IF. Deletion of the carboxyl terminus from this clone resulted in protein that still appeared capable of associating with desmosomes but not interacting with IF networks. As the amino terminus appeared to be dispensable for IF interaction, we made finer deletions in the carboxyl terminus of DP based on blocks of sequence similarity with the related molecules bullous pemphigoid antigen and plectin. We found a sequence at the very carboxyl terminus of DP that was necessary for coalignment with and disruption of keratin IF but not vimentin IF. Furthermore, the coalignment of specific DP proteins along keratin IF but not vimentin IF was correlated with resistance to extraction by Triton. The striking uncoupling resulting from the deletion of specific DP sequences suggests that the carboxyl terminus of DP interacts differentially with keratin and vimentin IF networks.

UNDERSTANDING the mechanisms by which the cytoskeleton attaches to the plasma membrane is fundamental to understanding how cells maintain their shape, regulate movement and adhesive potential, and even process signals originating at the plasma membrane (Green and Jones, 1990; Geiger and Ginsberg, 1991; Luna and Hitt, 1992). Specific cell–cell and cell–substrate junctions, prominent in epithelia, exist for both actin and intermediate filament (IF)[1] networks. Desmosomes and hemidesmosomes act as attachment points for IF at cell–cell and cell–substrate borders, respectively (for reviews see Schwarz et al., 1990; Green and Jones, 1990; Buxton and Magee, 1992; Garrod, 1993), while corresponding attachment points for actin filaments are adherens junctions and focal contacts (for reviews see Geiger and Ginsberg, 1991; Luna and Hitt, 1992; Tsukita et al., 1992). Of the cell–cell junctions, although desmosomes and adherens junctions are functionally quite distinct, the dissection of each junction at the molecular level has demonstrated several similarities. Both junctions contain cadherin-like cell–cell adhesion molecules (Buxton and Magee, 1992; Geiger and Ayalon, 1992), which presumably interact through their cytoplasmic tails with cytoplasmic molecules of the adhesion complex (Nagafuchi and Takeichi, 1988; Troyanovsky et al., 1993). These molecules of the junctional plaque are proposed to link and/or stabilize the interaction of cell surface adhesion molecules with cytoskeletal filaments. Other than plakoglobin, which is found in both junctions (Cowin et al., 1986), the plaque components have thus far been found to be unique to each junction. These junction-specific plaque components are thought to be responsible for the specificity of interaction of a given junction with a particular cytoskeletal element.

The ascribed function of individual components of each junction has been based primarily on protein–protein interactions. However, a detailed understanding of many of the

1. *Abbreviations used in this paper*: BPA, bullous pemphigoid antigen; DP I and II, desmoplakin I and II; ECL, enhanced chemiluminescence; IF, intermediate filaments.

protein-protein interactions within each junction and with specific cytoskeletal filament systems is still lacking. Standard biochemical techniques such as immunoprecipitation have been used successfully to demonstrate, for example, that E-cadherin from adherens junctions and desmoglein from desmosomes associate with the catenins and plakoglobin, respectively (Korman et al., 1989; Ozawa et al., 1989). However, these techniques have not been as successful at determining other junctional protein-protein interactions, particularly between desmosomal molecules (O'Keefe et al., 1989; Pasdar et al., 1991). While biochemical evidence for protein-protein interactions between desmosomal plaque molecules has been difficult to obtain, perhaps due to the insolubility of the desmosome, predicted protein structures based on cloned cDNA sequences have provided insights into potential interactions. This has been especially true for understanding the function of desmoplakin I and II (DP I and II).

DP I and II are two highly related phosphoproteins (Mueller and Franke, 1983) that are the most abundant constituents of the desmosomal plaque. Based on previous structural predictions (Green et al., 1990; Green et al., 1992; Virata et al., 1992) and biochemical analysis (O'Keefe et al., 1989), DP I is thought to be a homodimer with a central rod domain composed of an α helical coiled-coil flanked by amino and carboxyl-terminal globular domains. DP II is thought to be derived from a spliced DP mRNA resulting in a molecule with a shortened rod domain (Green et al., 1990). The carboxyl terminus of DP was predicted to be a potential site for ionic interaction with IF based on the similar periodicities of acidic and basic residues found in the carboxyl terminus of DP and the 1B rod domain of IF (Green et al., 1990). DP is a member of a family of proteins that includes 230 kD bullous pemphigoid antigen (BPA), which is located in the cytoplasmic plaque of the hemidesmosome, and plectin, an intermediate filament associated protein (Tanaka et al., 1991; Sawamura et al., 1991; Wiche et al., 1991; Green et al., 1992). Comparisons based on cDNA sequences indicated that BPA and plectin have an α helical coiled-coil central rod domain as does DP, though each has unique features. The flanking amino and carboxyl-terminal globular domains exhibit large scale sequence similarity among all three family members. Like DP, BPA and plectin contain similar subdomains within their respective carboxyl termini that are comprised of a series of 38-residue repeats. In addition, the most carboxyl-terminal tails of these molecules, downstream of the 38-residue repeats, show strong similarity (Green et al., 1992). Since plectin is a known intermediate filament associated protein (Foisner et al., 1988), and DP and BPA have been hypothesized to be associated with IF (Steinert and Roop, 1988), it is possible that similar regions in all three molecules could be important for their interaction with IF.

Previously, we tested the hypothesis that the carboxyl terminus of DP could interact with IF networks by transfecting cDNAs encoding specific domains of DP into COS-7 and NIH-3T3 cells (Stappenbeck and Green, 1992). The ectopically expressed carboxyl terminus of DP specifically coaligned with and disrupted cytoplasmic IF networks in these cell lines. These results suggest that DP can interact with IF in vivo, although the possibility that accessory or linking proteins are required has not been ruled out. In the current study, an investigation of the role of the amino terminus of DP indicates that, although this domain is apparently needed for localization to desmosomes, it is not required for interaction with IF networks. In addition, finer deletions have now been made within the carboxyl terminus of DP to identify regions responsible for the apparent interaction of the complete carboxyl terminus of DP with IF. The deletions have been constructed based on regions of similarity in DP, plectin, and BPA. These finer deletions define a region in the carboxyl terminus of DP that specifies the interaction with keratin versus vimentin IF networks.

Materials and Methods

Cell Culture

COS-7 African Green monkey kidney cells and NIH-3T3 mouse fibroblasts were cultured in DME. HeLa human epitheloid carcinoma cells and HaCaT human immortalized epidermal cells (kindly provided by Dr. John Stanley, Dermatology Branch, NCI, NIH and Dr. Norbert Fusenig, German Cancer Research Center, Heidelberg, Germany) were cultured in MEM. All lines were supplemented with 10% FCS and 100 U/ml penicillin and 100 μg/ml streptomycin.

Construction of Expression Vectors

Construction of cDNA Clone Encoding Human Full-length DP I

(a) Construction of a Fragment Containing the 3' Portion of the DP I Coding Sequence Linked to Human c-myc Tag. A 1,631-bp StyI/HindIII fragment from plasmid clone p31 (Green et al., 1990) was subcloned into a StyI/HindIII (partial)-digested p90 clone (Virata et al., 1992). A 4,872-bp KpnI/BglII fragment from pDP.ΔN (Stappenbeck and Green, 1992) containing most of the 3' portion of the DP I coding region, a 33-bp *c-myc* epitope tag, and an engineered TAG stop codon- Kpn I sequence was then subcloned into the KpnI/BglII (partial)-digested p31-p90 plasmid to produce plasmid pl84, which comprised the 3' 6,640 bp of the DP I coding sequence.

(b) Construction of a Fragment Containing the 5' Portion of the DP I Coding Sequence. The 989-bp EcoRI fragment of clone pl05 (Virata et al., 1992) was first reinserted into a pKS$^+$/Bluescript vector (Stratagene, La Jolla, CA) in the reverse orientation. A 1,033-bp KpnI/StyI fragment from clone pl30 (Virata et al., 1992) was then subcloned into the "reversed pl05" plasmid at the KpnI/StyI sites to produce a construct, pl81. A 622-bp PCR product, containing a 5' portion of clone pl50 (Virata et al., 1992) with an engineered SacII restriction site, was generated and then ligated into a PCR-1000 cloning vector, according to TA cloning kit instructions (Invitrogen, San Diego, CA). The sequence of the resulting plasmid, pl91, was later confirmed by double-stranded DNA sequencing. A 576-bp SacII/AccI fragment of pl91 was then subcloned into the SacII/AccI (partial)-digested pl81 to produce the construct, p200.

(c) Completion of DP I cDNA Construction and Insertion into Expression Vector. A 6.5-bp BfrI/KpnI fragment from pl84 was subcloned into p200. The 8-kb SacII/KpnI insert of the resulting plasmid p215 was later isolated and blunt-end ligated into an expression vector driven by the CMV promoter (plasmid p271, or pDP.NΔ194; White and Cipriani, 1989). The remaining cDNA was isolated by screening of a human keratinocyte LambdaZapII cDNA library (Tanaka et al., 1991). Overlapping phage clones were isolated and subcloned into a Bluescript vector for sequence analysis. The remaining DP cDNA which included a short sequence segment not previously published (revised GenBank accession #M77830) was subcloned upstream of the DP cDNA of p271.

Addition of 6 c-myc Tag Clone. Previously, a PmaCI site was engineered 5' to the original *c-myc* tag, which was located at the 3' end of the cDNA for DP (Stappenbeck and Green, 1992). A clone containing the six tandem repeats of the *c-myc* tag (kindly provided by Dr. Joe Gall, Carnegie Institute, Baltimore, MD) was digested with ApaI and PstI and blunt ended using T_4 DNA polymerase. This DNA fragment was then blunt end ligated into the PmaCI site of the full-length clone of DP previously tagged with one *c-myc*. This manipulation retained the reading frame of the DP cDNA.

Construction of Deletion Constructs in Figure 1. All deletions were generated using primers (Northwestern Biotechnology Research Service

Facilities, Chicago, IL) in PCR reactions (for details see Stappenbeck and Green, 1992) that added appropriate start or stop codons along with restriction sites that aided in routine subcloning.

DNA Transfections. Plasmid DNA was purified using QIAGEN anion exchange columns (QIAGEN, Inc., Chatsworth, CA) and was transfected into all cultured cells by calcium phosphate precipitation followed by a 15% glycerol shock. All experiments were analyzed 24–30-h postglycerol shock.

Preparation and Immunoblotting of Whole Cell Extracts

Whole-cell extracts were prepared 24 h postglycerol shock as described by Green et al. (1991). Protein content was determined by the method of Bradford (1976). Samples of 40 μg were loaded and run on SDS–polyacrylamide gels (6.5%). The gels were transferred to nitrocellulose and immunoblotting was performed as previously described (Angst et al., 1990). Primary antibodies used were a 1:2,000 dilution of an affinity-purified rabbit polyclonal, NW 6, directed against a fusion protein of the carboxyl terminus of DP (Angst et al., 1990); a 1:500 dilution of a mouse monoclonal, DPI-2.17, directed against DP I (Cowin et al., 1985); a 1:500 dilution of a mouse monoclonal, V9, directed against vimentin (Sigma Chemical Co., St. Louis, MO); a 1:1,000 dilution of a rabbit polyclonal anti–vimentin antiserum (ICN Immunobiologicals, Costa Mesa, CA); and a 1:1 dilution of a mouse monoclonal, 9E10.2, directed against a 11 amino acid fragment of human c-*myc* (Evans et al., 1985). A 1:1,000 dilution of peroxidase-coupled anti–rabbit or anti–mouse secondary antibodies (Kirkegaard and Perry Laboratories, Gaithersburg, MD) followed primary antibody incubation. Detection was performed by enhanced chemiluminescence (ECL; Amersham Corp., Arlington Heights, IL).

Immunofluorescence Labeling

For immunocytochemistry, cells were grown on glass coverslips and transfected as described above. Cells were washed in PBS and fixed in methanol (−20°C) for 2 min.

Primary antibodies used for staining were a mouse monoclonal 9E10.2 directed against an 11 amino acid fragment of human c-*myc* (Evans et al., 1985) used as undiluted hybridoma supernatant; a 1:50 dilution of a rabbit polyclonal, NW38, directed against a 57-mer synthetic peptide in the A subdomain of the carboxyl terminus of DP; a 1:20 dilution of a rabbit polyclonal anti–desmoglein antiserum (kindly provided by Orest Blaschuk, Royal Victoria Hospital, McGill University, Montreal, Canada); a 1:100 dilution of a mouse monoclonal, KS-B17.2, directed against K18 (Sigma Chemical Co., St. Louis, MO); a 1:10 dilution of a mouse monoclonal, V9, directed against vimentin (Sigma Chemical Co.); a 1:20 dilution of a mouse monoclonal DP-2.15 directed against DP I and II (Boehringer-Mannheim Biochemicals, Indianapolis, IN); and a 1:20 dilution of a rabbit polyclonal anti-vimentin antiserum (ICN Immunobiologicals, Costa Mesa, CA).

To visualize the primary antibody, appropriate fluorescein or rhodamine conjugated anti–rabbit or anti–mouse (Kirkegaard and Perry Laboratories) secondary antibodies were diluted 1:20 in PBS. Controls included incubation of fixed cells in conjugated secondary antibodies alone or with preimmune rabbit serum for polyclonal antibodies.

Triton Extraction of Transfected Cell Population

HeLa and 3T3 cells grown on coverslips were transiently transfected as previously described. 24-h postglycerol shock, transfected cells were incubated with 0.5% Triton X-100 in PBS for 15 min at room temperature with gentle agitation immediately before either the preparation of gel samples or immunofluorescence. Immunoblotting of gel samples was performed as described above. Resulting x-ray films exposed by ECL were analyzed by scanning densitometry using an UltroScan XL (LKB Instruments, Bromma, Sweden).

Results

Protein Expressed by a Tagged Full-length DP I Construct Colocalizes with Desmosomes in COS-7 Cells

To further investigate the role of specific DP sequences in the association with IF networks, and to determine which domain(s) of DP facilitate association with desmosomes at the cell surface, a 9.5-kb cDNA encoding full-length DP I was assembled from several overlapping phage clones (Green et al., 1990; Virata et al., 1992; Virata, M. L. A., E. A. Bornslaeger, and K. J. Green, unpublished results). This cDNA is predicted to encode a polypeptide of 334 kD, which includes 194 amino acids at the extreme amino terminus not previously reported (GenBank accession #M77830). The complete cDNA was subcloned into a mammalian expression vector driven by the CMV promoter (Fig. 1; White and Cipriani, 1989). To identify proteins encoded by transfected plasmids, a 33-bp sequence encoding a portion of c-*myc* was added to the 3′ end of the cDNA (Evans et al., 1985). The c-*myc* tag in this position had been used previously for amino terminal deletions of DP without any apparent deleterious effects (Stappenbeck and Green, 1992). In the case of the full-length DP cDNA, however, c-*myc*–tagged DP protein was not detected by immunoblotting whole cell lysates of any transiently transfected tissue culture cell lines that were tested (Fig. 2, lane *7* for one example in COS-7 cells). This was also true for other constructs that included the amino terminus of DP (data not shown). In contrast, DP.ΔN, which did not include the amino terminus (Fig. 1), was easily detected on immunoblots (Fig. 2, lane *8*; Stappenbeck and

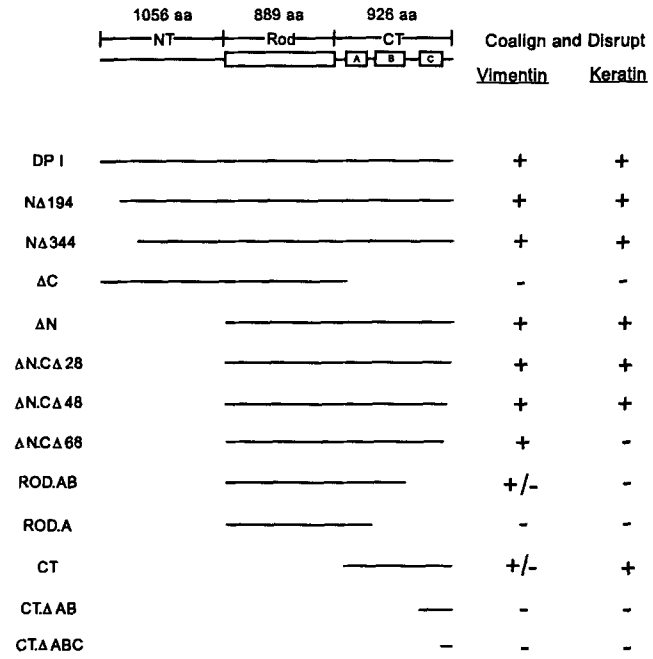


Figure 1. Summary of deletion analysis results. Schematic diagram of full length DP I showing the predicted domains, including the amino terminus (*NT*), the rod domain, and the carboxyl terminus (*CT*). The carboxyl terminus includes three subdomains, A, B, and C, which are composed of series of 38-residue repeats. The extreme carboxyl terminus (downstream of the C domain) is composed of 68 amino acids. Below the diagram are representations of the deletion constructs. Also shown is a summary of the immunofluorescence analysis to determine whether coalignment with and disruption of IF networks resulted from the overexpression of each protein in tissue culture cells. "−" indicates no coalignment with or disruption of a particular IF network in >95% of transfected cells examined. "+" indicates coalignment with or disruption of a particular IF network in >95% of transfected cells examined.

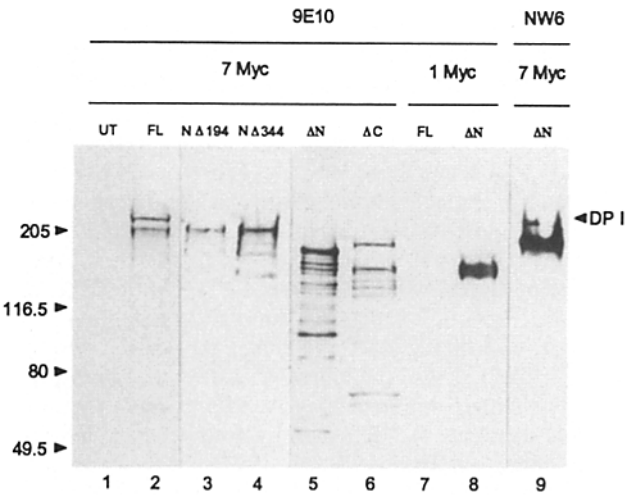


Figure 2. Detection of truncated and full length DP expressed in COS-7 cells by immunoblotting. COS-7 cells were transfected with the indicated DP construct and were lysed in urea sample buffer 24 hours post glycerol shock. 40 μg of total protein (except for lane *5* in which only 4 μg of total protein was used) from COS-7 cells transfected with no DNA (*UT*, lane *1*), pDP.7 myc (*FL*, lane *2*), pDP.NΔ194 (*NΔ194*, lane *3*), pDP.NΔ344.7myc (.7myc, lane *4*), pDP.ΔN.7 myc (*ΔN*, lanes *5* and *9*), pDP.ΔC.7 myc (*ΔC*, lane *6*), pDP.myc (lane *7*), and pDP.ΔN.myc (lane *8*) were loaded and run on a 6.5% SDS-polyacrylamide gel. The gel was transferred to nitrocellulose and lanes *1–8* were reacted with a mouse monoclonal directed against an 11-amino acid epitope of c-myc (9E10.2). Lane *9* was reacted with a rabbit polyclonal antibody directed against the carboxyl terminus of DP (*NW 6*). The position of both exogenous (lane *2*) and endogenous DP I (lane *9*) is indicated.

Green, 1992). However, a few cells transfected with full-length DP did display positive staining by immunofluorescence with the 9E10.2 antibody directed against the c-*myc* epitope tag. This suggested that constructs including the amino terminus of DP were expressed in the transfected cell population, but at levels that were not detectable by immunoblotting.

To increase the sensitivity of detection, a plasmid encoding a tandemly repeated series of six c-*myc* tags (Roth et al., 1991) was subcloned in frame 5′ of the original c-*myc* tag in the full-length DP clone, thus creating a clone encoding a seven c-*myc*–tagged full-length DP. This construct encoded protein that was detectable by immunoblotting using the 9E10.2 antibody. Using this construct with the extended tag also resulted in at least a one hundred fold increase in the number of transfected cells in the population that were positive as assessed by immunofluorescence. On immunoblots, two major bands were observed, one which migrated at the expected molecular weight of DP I, and another which migrated at a lower molecular weight (Fig. 2, lane *2*). Lower molecular weight bands were also detected in samples of all other seven c-*myc*–tagged DP truncation constructs (Fig. 1 and 2, lanes *3–6*; note that lane *5* was loaded with only 4 μg of total cellular protein as compared to 40 μg in all other lanes). It is possible that these lower molecular weight bands may have been generated as a result of increased susceptibility to proteolysis in the presence of the seven c-*myc* tag. However, the sensitivity of the 9E10.2 antibody for the multiple c-*myc* tags must at least in part be responsible for the detection of these apparent breakdown products; when ten times the amount of lysate of the seven c-*myc*–tagged DP.ΔN was immunoblotted using an antibody to DP (NW 6), none of the breakdown products were observed (Fig. 2, compare lanes *5* and *9*). Most importantly, protein generated from seven c-*myc* tagged constructs appeared to be functional based on subcellular localization (as discussed in detail below). In addition, for a given construct, the presence or absence of single or multiple c-*myc* tags did not appear to affect the subcellular localization of the expressed products.

The subcellular localization of the seven c-*myc* tagged full-length DP was determined by immunofluorescence of transiently transfected COS-7 cells using the 9E10.2 antibody. COS-7 cells were chosen because the percentage of positive cells in cultures transfected with constructs including the amino terminus was the highest in this cell line as assessed by immunofluorescence (although only 1–2%). For unknown reasons, the percentage of positive cells in cultures transfected with constructs including the amino terminus was consistently lower than in cultures transfected with constructs of DP lacking the amino terminus (30–40%). In addition, COS-7 cells assemble desmosomes when grown at high density as observed by electron microscopic analysis (T. Stappenbeck, unpublished observation). Thus, this cell line was an appropriate choice for investigating the effects of specific mutations on the ability of DP to associate with desmosomes. It should be noted, however, that desmosomes are not expressed at all cell–cell borders among a given population of COS-7 cells.

Cells transfected with plasmids encoding full-length DP displayed two predominant patterns of fluorescence. In approximately 90% of the positive cells, tagged full-length DP coaligned with and disrupted IF networks, as will be discussed below. In approximately 10% of the positive cells, the 9E10.2 antibody revealed punctate staining at cell–cell borders much like that of COS-7 cells reacted with DP antibodies (Fig. 3, *A* and *B*). A proportion of these cells displayed only 9E10.2 positive spots at cell–cell borders (Fig. 3 *A*), while others displayed 9E10.2 positive cytoplasmic aggregates in addition to the cell–cell border staining (Fig. 3 *C*). The localization of 9E10.2 positive spots at cell–cell borders suggested that the seven c-*myc*–tagged full-length DP was capable of incorporating into or associating with the cytoplasmic face of endogenous desmosomes in COS-7 cells. To demonstrate this more clearly, cells transfected with full-length–tagged DP were double labeled with 9E10.2 and an antibody directed against the transmembrane desmosomal glycoprotein, desmoglein (Fig. 3, *C–F, Dsg*). High magnification of cell–cell borders with 9E10.2 positive spots revealed colocalization with Dsg positive spots (Fig. 3, *E* and *F*). It appeared that each 9E10.2 spot at cell–cell borders colocalized with a corresponding Dsg spot. This result indicates that the tagged DP apparently associates with desmosomes and does not merely form aggregates at the cell surface.

Most 9E10.2 positive spots at cell–cell borders colocalized with DP positive spots (Fig. 3. *A* and *B*, *arrows*); however, the relative intensities of the two were often different. In some cases DP spots reacted only faintly with 9E10.2, which may indicate desmosomes with little or no association of tagged DP. Surprisingly, a proportion of the 9E10.2 spots reacted only faintly, or not at all with antibodies directed

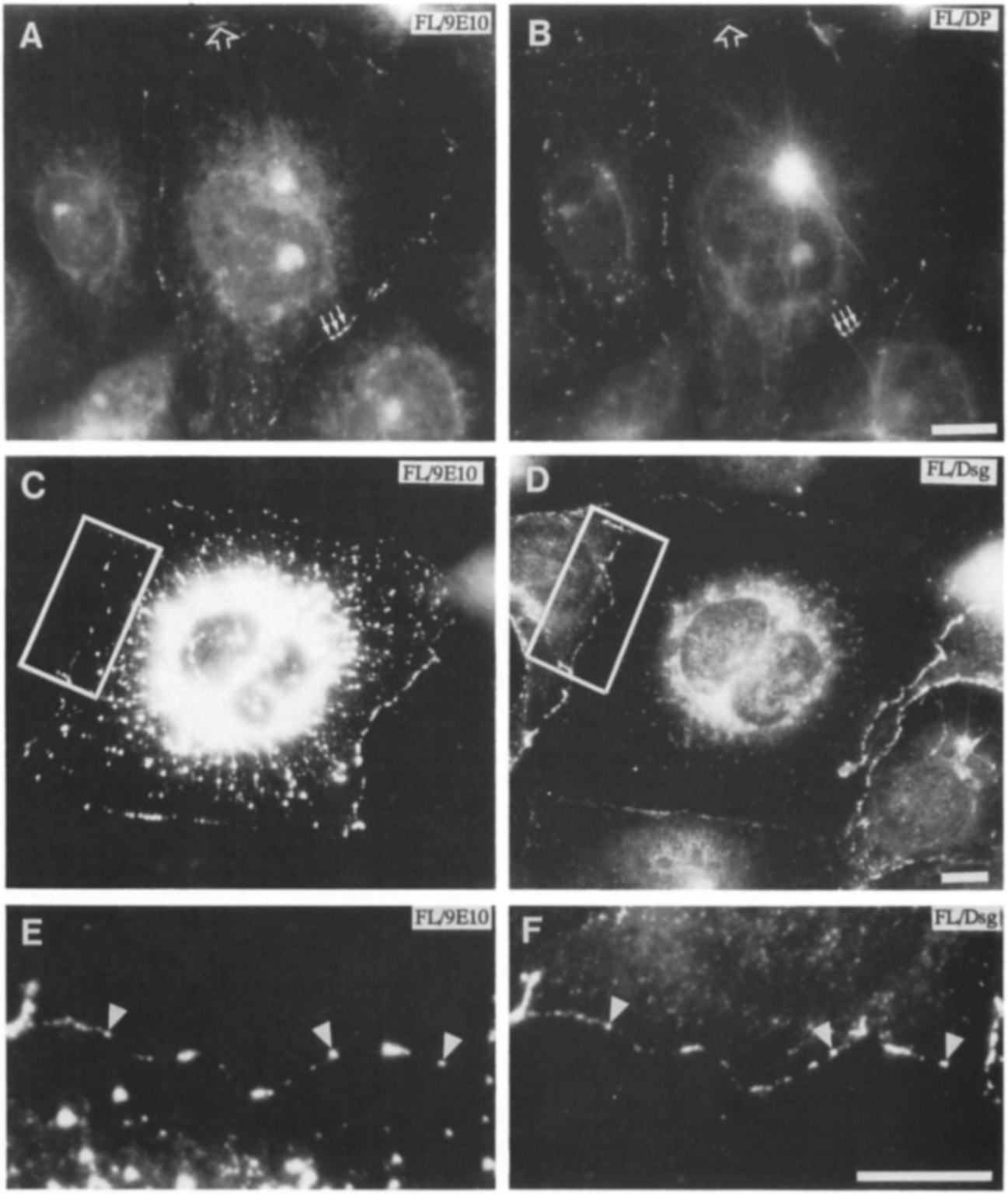


Figure 3. Indirect double label immunofluorescence of COS-7 cells transfected with pDP.7myc (full-length DPI, FL). 24 h after glycerol shock A, C, and E were reacted with the mouse monoclonal, 9E10.2, directed against the c-*myc* tags. *B* was reacted with the rabbit polyclonal antibody, NW 38, directed against a peptide in the carboxyl terminus of DP. *D* and *F* were reacted with a rabbit polyclonal antibody directed against bovine desmoglein (*Dsg*). Small solid arrows in *A* and *B* indicate colocalization of 9E10.2 and DP staining at cell–cell borders. Outlined arrows in *A* and *B* indicate 9E10.2 specific staining at cell–cell borders. The rectangle in *C* corresponds to *E* and the rectangle in *D* corresponds to *F* (each rectangle is rotated 70° clockwise). Arrowheads in *E* and *F* indicate examples of 9E10.2 and Dsg spots that colocalize at cell-cell borders, suggesting that tagged DP can associate with desmosomes. Labels in upper right of each panel specify DP construct/antigen recognized by primary antibody. Bars, 10 μm.

against DP. One possible explanation for this result is the greater sensitivity of the 9E10.2 antibody for the seven c-*myc* tag on exogenous DP relative to the sensitivity of the DP antibody for both endogenous and exogenous DP. The 9E10.2 specific spots may represent nascent desmosomes in the process of recruiting newly synthesized DP, most of which was tagged.

Sequences in the Amino Terminus of DP Are Necessary for Colocalization with Desmosomes

Due to the presence of apparent breakdown products in COS-7 cells transiently transfected with full-length DP, it is possible that some of the protein detected by 9E10.2 at cell surfaces actually consisted of smaller fragments of DP that lacked part of the amino terminus. To address this issue and also to test if the complete amino terminus of DP is necessary for localization to cell–cell borders, amino terminal truncations from full-length DP were constructed, the smallest of which were 194 and 344 amino acids (Fig. 1). As with the full-length DP construct, both NΔ194 and NΔ344 were only detected in transiently transfected COS-7 cells when tagged with the seven c-*myc* epitope. Immunoblot analysis of cells expressing these constructs revealed, in addition to intact tagged protein at the expected molecular weight, apparent breakdown products (Fig. 2, lanes *3* and *4*). Both of the intact protein products seemed to have similar electrophoretic mobilities despite the difference of 150 amino acids. However, both proteins have greater electrophoretic mobility than full-length DP I. The actual molecular weight of each of these constructs could not be calculated accurately, as molecules in this gene family run abberantly on SDS-PAGE gels (i.e., size estimates for DP, BPA, and plectin were all significantly lower than the predicted molecular weights based on cDNA sequence).

Using the 9E10.2 antibody, immunofluorescence analysis of COS-7 cells expressing either NΔ194 or NΔ344 revealed similar patterns. In both cases, filamentous arrays were typically observed (Fig. 4, *A* and *B* for NΔ194; data not shown for NΔ344) that were similar to those seen in cells expressing DP.ΔN, lacking the entire amino terminus (Stappenbeck and Green, 1992). Double label immunofluorescence with 9E10.2 and antibodies directed against either keratin or vimentin IF revealed that these truncated DP proteins colocalized with both of these IF systems (not shown). While this same pattern was observed in a proportion of cells expressing full-length DP (see below), punctate staining at cell–cell borders has only been observed in cells expressing full-length DP and not in cells expressing any of the amino terminal deletions. Double label immunofluorescence with 9E10.2 and an antibody to Dsg of cells transfected with pNΔ194 revealed that the 9E10.2 filamentous pattern adjacent to cell–cell borders did not appear to significantly colocalize with the punctate Dsg positive spots along the plasma membrane (Fig. 4, *A–D*). While incorporation of truncated DP into desmosomes where IF terminate cannot be ruled out, in some cases, there appeared to be a gap in the filamentous 9E10.2 pattern that corresponded to a Dsg positive spot (Fig. 4, *C* and *D*, *arrows*). Taken together, this evidence indicates that truncations as small as 194 amino acids from the amino terminus of DP compromise the ability of DP to efficiently associate with desmosomes. This evidence further suggests that the major putative breakdown product observed in cells transfected with plasmids encoding full-length DP (Fig. 2, lane *2*) may not be capable of associating with desmosomes efficiently if at least 194 amino acids had been removed from its amino terminus. Therefore, the punctate cell–cell border staining of 9E10.2 in cells transfected with full length DP is most likely intact tagged protein.

The Carboxyl Terminus of DP Is Dispensable for Localization to Desmosomes but Is Necessary for Coalignment with, and Disruption of, IF

To test if the apparent incorporation of full-length–tagged DP into desmosomes was dependent on the presence of the carboxyl terminus, a construct encoding a seven c-*myc*–tagged deletion of the carboxyl-terminal domain of full-length DP (pDP.ΔC; Fig. 1) was transiently transfected into COS-7 cells (see Fig. 2, lane *6* for immunoblot). 9E10.2 staining revealed large cytoplasmic aggregates (see below) as well as punctate staining at cell–cell borders (Fig. 4 *E*). As with full-length DP, double label immunofluorescence with 9E10.2 and an antibody to Dsg revealed that the spots at cell–cell borders colocalized with Dsg positive spots (Fig. 4, *E* and *F*). Thus, the absence of the carboxyl terminus did not apparently have a deleterious effect on the association of DP.ΔC with desmosomes.

Previously, we demonstrated that the carboxyl terminus of DP was necessary and sufficient for coalignment with and disruption of IF within the context of the amino terminal deletion construct (Stappenbeck and Green, 1992). We wished to test if the same would hold true within the context of full-length DP. As mentioned above, approximately 90% of cells expressing tagged full-length DP displayed a pattern of 9E10.2 staining that was either in the form of filaments that colocalized with intact vimentin and keratin IF (not shown) or large perinuclear aggregates (Fig. 5 *A*) that colocalized with disrupted vimentin (Fig. 5 *B*) and keratin (not shown) networks. The coalignment with and disruption of both IF networks was similar to previous observations made with both DP.ΔN and DP.CT (Stappenbeck and Green, 1992). In contrast to the full-length DP protein, DP.ΔC formed 9E10.2 reactive cytoplasmic aggregates that did not colocalize with or disrupt either vimentin or keratin IF networks by double label immunofluorescence (Fig. 5, *C* and *D*). As a control for the presence of the multiple c-*myc* tags, an otherwise identical, untagged DP.ΔC construct was created. Immunofluorescence of transfected cells using an antibody to DP revealed similar large aggregates in a few of the highest expressing cells. Again, the IF networks in these cells were unaffected (data not shown), confirming that the carboxyl terminus is required for interaction with IF networks.

These results also suggest that the carboxyl terminus of DP is not required for the association of DP with desmosomes. In fact, a greater percentage of cells expressing DP.ΔC exhibited tagged protein that colocalized with Dsg at cell–cell borders (~40% of transfected cells) as compared with cells expressing full-length DP (~10% of transfected cells). A possible explanation for this result is that the absence of the carboxyl terminus may free the ectopically expressed DP.ΔC from interaction with IF in the cytoplasm. Therefore this

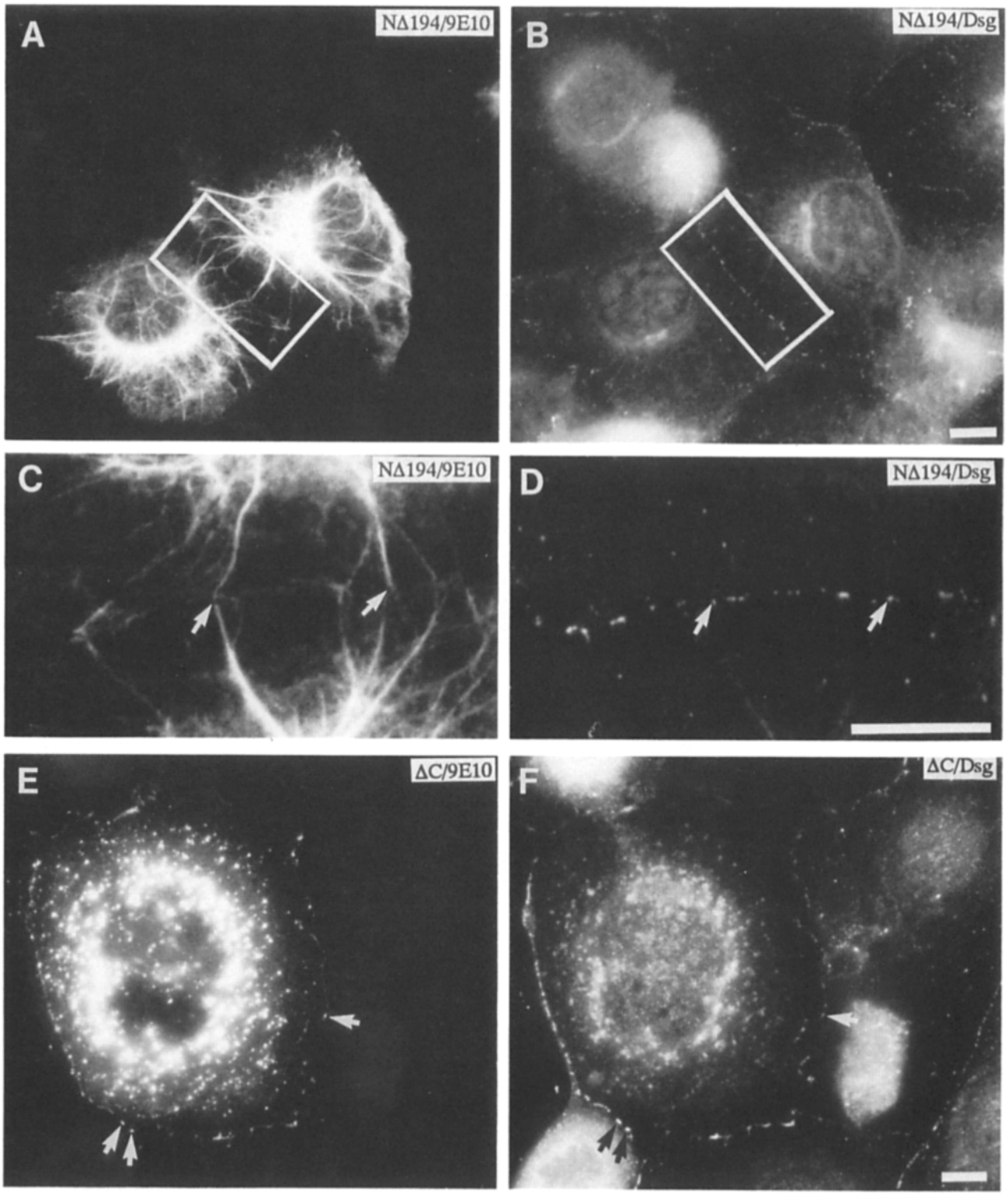


Figure 4. Indirect double label immunofluorescence of COS-7 cells transfected with pDP.NΔ194 (*A–D*) or pDP.ΔC (*E* and *F*). 24 h after glycerol shock *A*, *C*, and *E* were reacted with the mouse monoclonal, 9E10.2, directed against the c-*myc* tag. *B*, *D*, and *F* were reacted with a rabbit polyclonal antibody directed against bovine desmoglein (*Dsg*). The rectangle in *A* corresponds to *C* and the rectangle in *B* corresponds to *D* (each rectangle is rotated 120° clockwise). Arrows in *C* and *D* indicate Dsg spots located in gaps between the filamentous 9E10.2 pattern. Arrows in *E* and *F* indicate 9E10.2 and Dsg spots along cell–cell borders that colocalize, suggesting that DP.ΔC can associate with desmosomes. Bars, 10 μm.

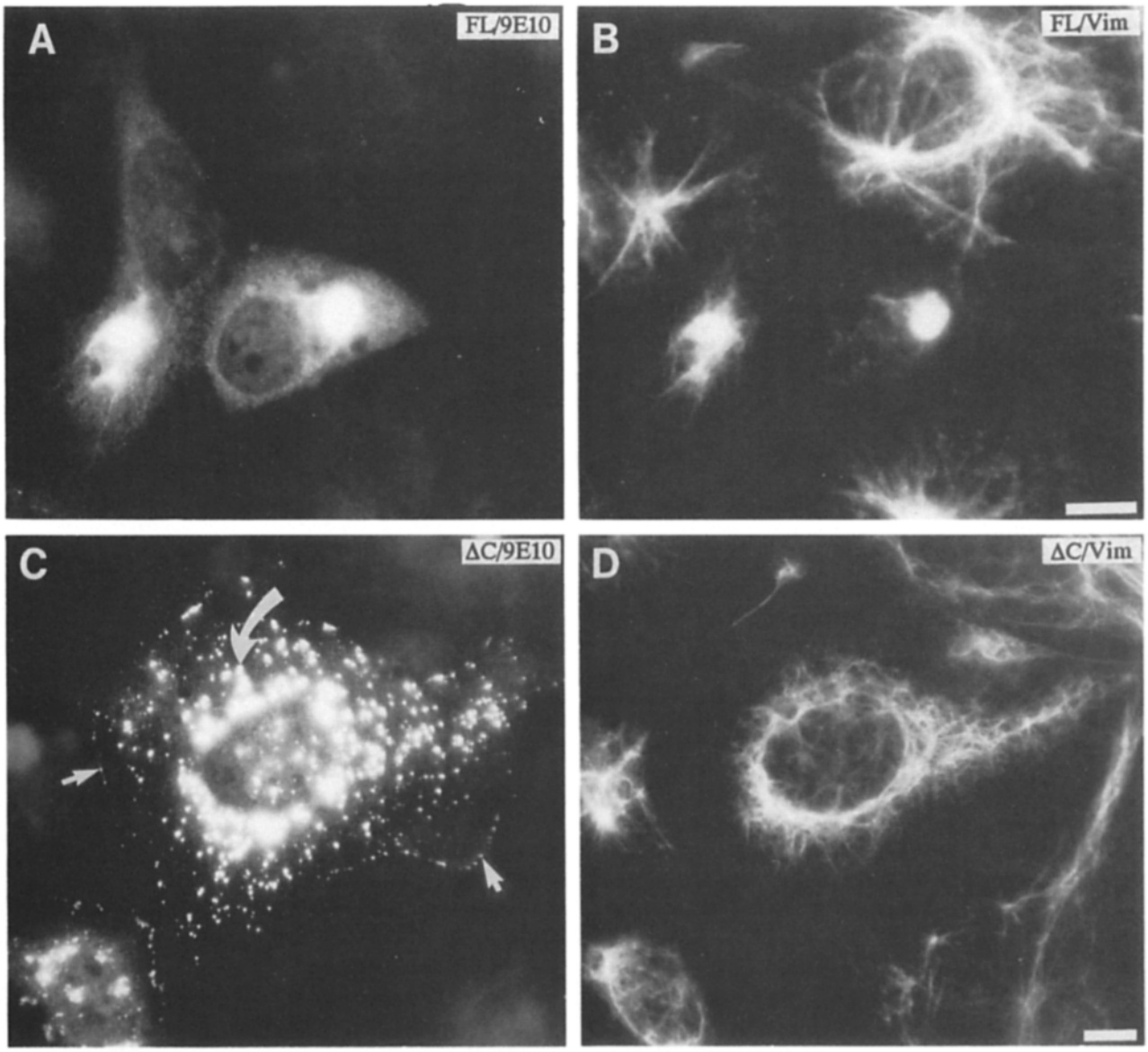


Figure 5. Indirect double label immunofluorescence of COS-7 cells transfected with pDP.7myc (*A* and *B*; full length, *FL*) or pDP.ΔC.7myc (*C* and *D*). 24 h after glycerol shock, *A* and *C* were reacted with the mouse monoclonal, 9E10.2, directed against the c-*myc* tags. *B* and *D* were reacted with a rabbit polyclonal to vimentin. Short arrow in *C* indicates 9E10.2 specific staining at cell–cell borders. Curved arrow in *C* indicates 9E10.2 specific staining of non-IF–associated aggregates in the cytoplasm. Bars, 10 μm.

protein may have been more readily available to associate with desmosomes.

Deletion Analysis of the DP Carboxyl Terminus Reveals a Specific Sequence Necessary for Coalignment with the Keratin IF Network

Previously, we determined that the carboxyl terminus of DP coaligned with and disrupted both vimentin and keratin IF when overexpressed in COS-7 cells (Stappenbeck and Green, 1992). Finer deletions of the carboxyl terminus were made in order to further define how this interaction might take place (Fig. 1). Since the amino terminus is apparently dispensable for interaction with IF, these deletions were made within the context of DP.ΔN instead of full-length DP to facilitate overexpression. The size and relative expression level of each truncated protein were determined by immunoblotting whole cell lysates of transiently transfected HeLa cells. In each case, the expressed polypeptide migrated at the predicted molecular weight on SDS-PAGE (Fig. 6). In addition the exogenous DP polypeptides were all expressed at similar levels, each of which greatly exceeded the amount of endogenous DP. The deletion constructs were transfected into several different cell lines to test the ability of each truncated protein to coalign with and disrupt vimentin (3T3), keratin (HaCaT), or both types of IF in the same cell (HeLa). These deletions of the carboxyl terminus were not c-*myc* tagged; transfected cells overexpressing truncated proteins could easily be identified by cytoplasmic DP staining distinct from the small amount of the endogenous DP present at cell–cell borders in either HeLa or HaCaT cells. As in COS cells, DP.ΔN coaligned with (Fig. 7, *A* and *B*) and disrupted

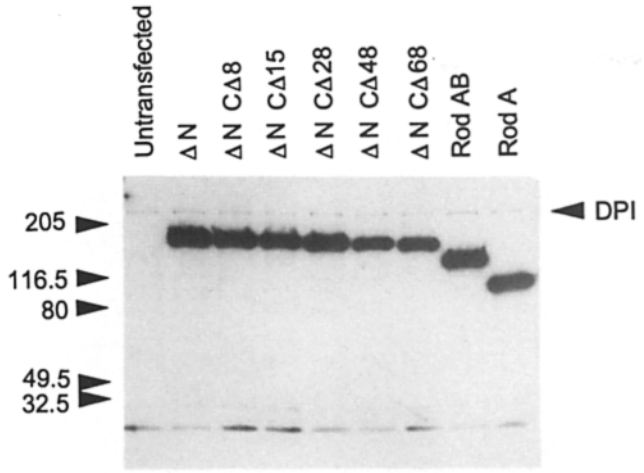


Figure 6. Detection of truncated DP expressed in HeLa cells by immunoblotting. HeLa cells were transfected with the indicated DP construct and were lysed in urea sample buffer 24 h postglycerol shock. 40 μg of total protein from HeLa cells transfected with no DNA (lane *1*), pDP.ΔN (lane *2*), pDP.ΔN.CΔ8 (lane *3*), pDP.ΔN.CΔ15 (lane *4*), pDP.ΔN.CΔ28 (lane *5*), pDP.ΔN.CΔ48 (lane *6*), pDP.ΔN.CΔ68 (lane *7*), pDP.RODAB (lane *8*), and pDP.RODA (lane *9*) were loaded and run on a 6.5% SDS-polyacrylamide gel. The gel was transferred to nitrocellulose and reacted with a mouse monoclonal to DP I (DP I 2.17). The position of endogenous DP I is noted with an arrow.

(Fig. 7, *C* and *D*) keratin IF and vimentin IF (not shown) in HeLa cells. Similar results were obtained for HaCaT and 3T3 cells (data not shown).

Deletion of the carboxyl-terminal 68 residues from DP.ΔN led to a striking uncoupling of the ability of this protein to coalign with and disrupt keratin IF versus vimentin IF. HeLa cells transfected with pDP.ΔN.CΔ68 and reacted with an antibody to DP usually exhibited a diffuse pattern, often with a juxtanuclear caplike structure (Fig. 8 *A*). In double label immunofluorescence using antibodies directed against DP and keratins, DP.ΔN.CΔ68 did not appear to significantly coalign with (Fig. 8, *A* and *B*) or disrupt keratin IF (Fig. 8, *C* and *D*). In contrast, double label immunofluorescence with antibodies directed against DP and vimentin revealed that this IF network was disrupted and organized into perinuclear aggregates that colocalized with DP (Fig. 8, *E* and *F*). These results were consistent with those from transfected 3T3 and HaCaT cells, which express only vimentin or keratin IF, respectively (data not shown). Smaller deletions from the carboxyl terminus of up to 48 amino acids did not reproduce this phenotype (Fig. 1), indicating that the amino acids 48–68 residues from the carboxyl terminus were necessary for the interaction of DP with keratin IF but were apparently dispensable for the interaction with vimentin IF.

While DP.ΔN.CΔ68 did not significantly coalign with keratin IF, it is possible that the diffuse staining masked a potential lower affinity interaction of this truncated DP with the keratin IF network. To address this issue, HeLa and 3T3 cells that were transfected with either pDP.ΔN or pDP.ΔN.CΔ68 were briefly incubated with 0.5% Triton in PBS, and then processed for immunoblotting and immunofluorescence. Vimentin was used as a loading control for immunoblotting experiments, as extraction of tissue culture cells with Triton does not significantly remove elements of the cytoskeleton such as vimentin (Fey et al., 1984). Treatment of transfected HeLa cells with Triton extracted approximately 80% of DP.ΔN.CΔ68, while under the same conditions little or no DP.ΔN was extracted (Fig. 9). As determined by immunofluorescence, DP.ΔN coaligned with keratin networks as in untreated cells. Unextracted DP.ΔN.CΔ68 protein was present in the form of aggregates and some filamentous staining that appeared to colocalize with vimentin but not keratin IF (data not shown). To test if the presence of the keratin IF network was responsible for the resistance to extraction of DP.ΔN in HeLa cells, the experiment was repeated in 3T3 cells, which contain only vimentin IF. In these cells, approximately 90% of both DP.ΔN and DP.ΔN.CΔ68 were extracted from transfected cells (Fig. 9). As determined by immunofluorescence, unextracted DP.ΔN and DP.ΔN.CΔ68 protein were present in the form of aggregates with disrupted vimentin and also along cabled vimentin IF (data not shown). These results indicate that even though DP.ΔN can coalign with both keratin and vimentin IF, only that associated with the keratin IF system was not significantly Triton extractable. This suggests that the interaction of DP with the keratin IF network may be of higher affinity as compared with the vimentin IF network. In summary, the presence of the carboxyl-terminal 68 residues of DP as well as keratin IF are necessary to prevent the detergent extraction of DP.ΔN from transfected tissue culture cells.

While the last 68 residues of DP are apparently necessary for the interaction of DP with keratin IF, are they sufficient? To test this, a construct was designed that included only the coding sequence for the carboxyl-terminal 68 residues of DP (DP.CT.ΔABC; Fig. 1). To localize this protein by immunoblotting and immunofluorescence, a sequence encoding a single c-*myc* tag was added at the 3′ end of the cDNA for this construct. 9E10.2 staining of HeLa or HaCaT cells transiently transfected with this construct revealed small cytoplasmic aggregates which did not coalign with or disrupt keratin IF (data not shown). It is possible that sequences immediately upstream of the 68 mer may also be necessary for this interaction. The remainder of the carboxyl terminus comprises three subdomains (A, B, and C) predicted on the basis of the grouping of 38-residue repeats and their linking sequences (Green et al., 1990). A protein encoded by a construct that contained the coding sequence for the C subdomain plus the 68-mer (DP.CT.ΔAB; Fig. 1) was likewise unable to coalign with and disrupt keratin IF (data not shown). These 68 carboxy-terminal amino acids therefore appeared to be necessary, but not sufficient for keratin IF interaction with DP. We cannot rule out the possibility, however, that the c-*myc* tag could have altered the conformation of CT.ΔABC or CT.ΔAB or that the conformation of these peptides were unlike the larger molecule (DP.ΔN).

Since the most carboxyl-terminal 68 amino acids were dispensable for the interaction of DP with vimentin IF, other portions of the DP carboxyl terminus must be required for this interaction. To determine the importance of the A, B, and C subdomains for the interaction with vimentin IF, each subdomain was deleted in succession (Fig. 1). As described previously, the removal of the carboxyl-terminal 68 amino acids did not affect the coalignment of this protein with vimentin IF when expressed in 3T3 cells (Fig. 10, *A* and *B*).

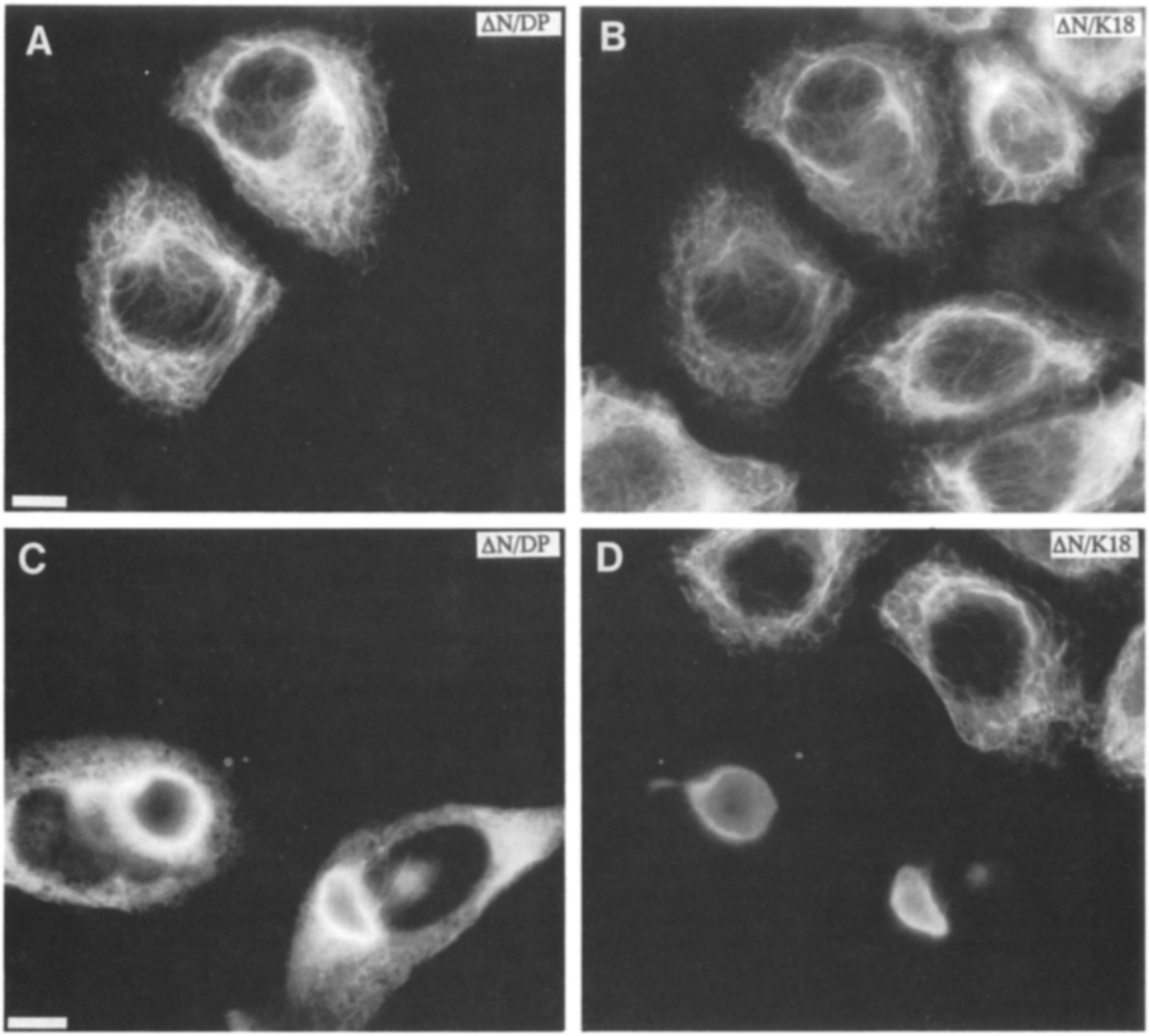


Figure 7. Indirect double label immunofluorescence of HeLa cells transfected with pDP.ΔN. 24 h after glycerol shock, *A* and *C* were reacted with a rabbit polyclonal to DP (NW 38) and *B* and *D* were reacted with a mouse monoclonal to keratin 18 (KS-B17.2). *A* and *B* demonstrate coalignment of DP.ΔN with keratin IF in cells that presumably express lower levels of DP.ΔN, while *C* and *D* demonstrate disruption of the keratin IF in cells that presumably express higher levels of DP.ΔN. Bars, 10 μm.

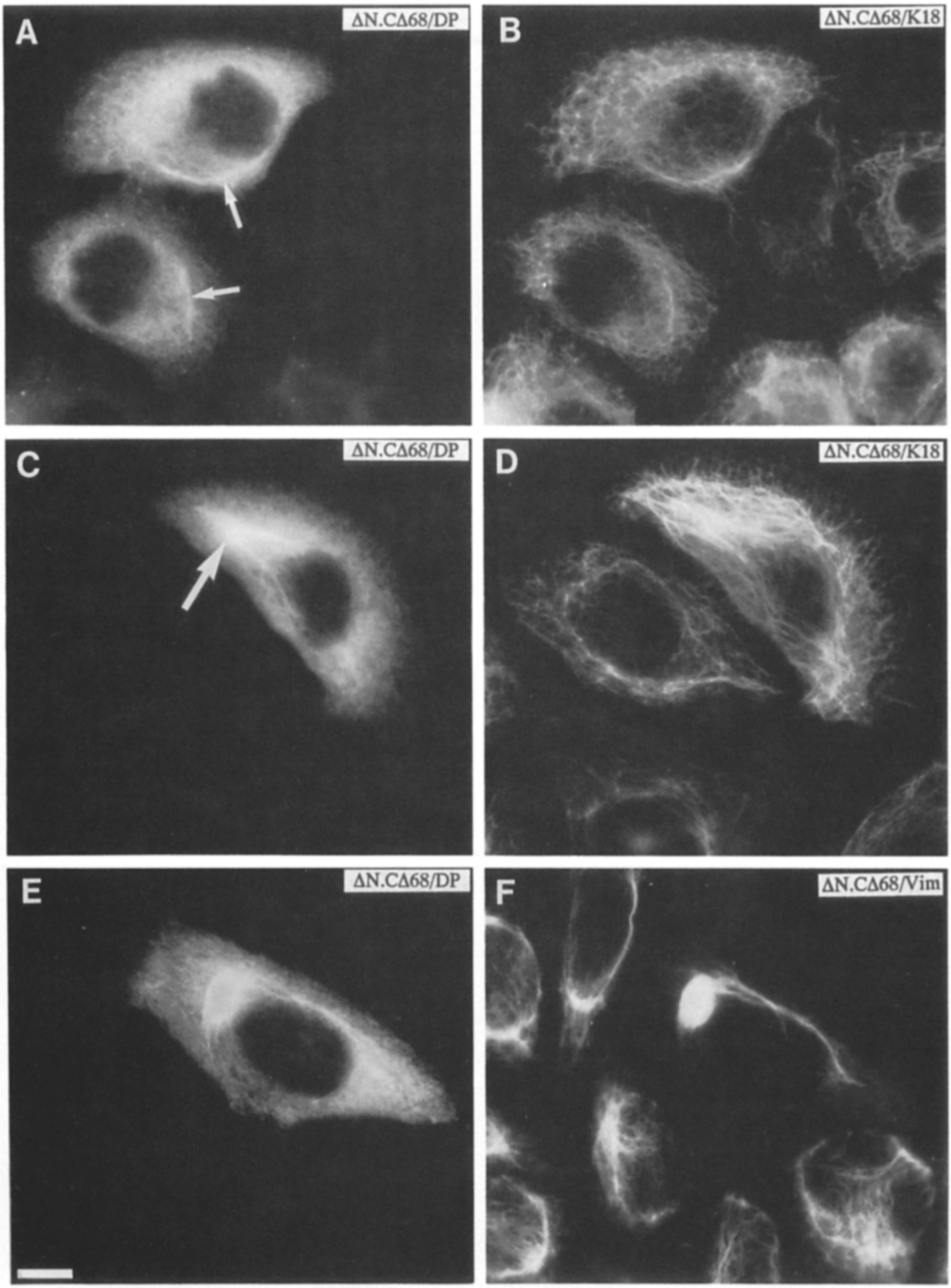
Removal of the C subdomain from DP.ΔN (ROD.AB) resulted in a truncated DP that could still partially coalign with and disrupt vimentin IF (Fig. 10, *C* and *D*). However, the alignment was not nearly as precise as with DP.ΔN or DP.ΔN.CΔ68 (see also Stappenbeck and Green, 1992). Subsequent removal of the B subdomain (ROD.A) resulted in the inability of this protein to either coalign with or disrupt vimentin IF in 3T3 cells (Fig. 10, *E* and *F*) or HeLa cells (not shown).

Discussion

The Amino Terminus of DP Is Necessary for Localization to Desmosomes but not for Interaction with Cytoplasmic IF Networks

We previously examined the localization of ectopically expressed domains of DP I (Stappenbeck and Green, 1992). We found that the carboxyl terminus alone or in combination

Figure 8. Indirect double label immunofluorescence of HeLa cells transfected with pDP.ΔN.CΔ68. 24 h after glycerol shock, *A*, *C*, and *E* were reacted with a rabbit polyclonal to DP (NW 38), *B* and *D* were reacted with a mouse monoclonal to keratin 18 (KSB17.2), and *F* was reacted with a mouse monoclonal to vimentin (V9). DP.ΔN.CΔ68 did not coalign with or disrupt keratin IF but did disrupt the vimentin IF. Arrows in *A* indicate positions of juxtanuclear caps that typically colocalize with disrupted vimentin IF as shown in *E* and *F*, while keratin IF in these cells are not disrupted. Arrow in *C* indicates aggregate of DP.ΔN.CΔ68 that did not result in the disruption of the keratin IF in this cell. Bar, 10 μm.


A
ΔN.CΔ68/DP
B
ΔN.CΔ68/K18
C
ΔN.CΔ68/DP
D
ΔN.CΔ68/K18
E
ΔN.CΔ68/DP
F
ΔN.CΔ68/Vim

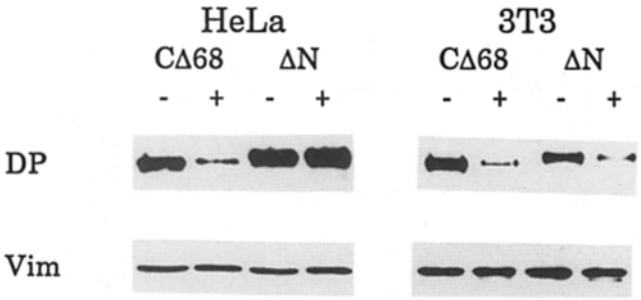


Figure 9. Immunoblot of HeLa and 3T3 cells transiently transfected with either pDP.ΔN or pDP.ΔN.CΔ68. 24-h postglycerol shock, cells were either incubated with PBS (−) or 0.5% Triton (+) for 15 min at room temperature. The remaining cytoskeleton was extracted in urea sample buffer. Vimentin, which is not extracted by this procedure, was immunoblotted as a loading control (the mouse monoclonal, V9 was used for HeLa extracts and a rabbit polyclonal was used for 3T3 extracts). Immunoblotting with a polyclonal antibody to DP revealed that 80% of DP.ΔN.CΔ68 was extracted from HeLa cells while DP.ΔN was not significantly extracted. Both DP.ΔN and DP.ΔN.CΔ68 were equivalently extracted from 3T3 cells, which do not contain keratin IF. This result was observed in three separate experiments.

with the rod domain coaligned with and disrupted IF networks. Neither of these domains, however, either alone or in combination, appeared to incorporate into desmosomes, suggesting that the amino terminal domain may be necessary for interaction with desmosomal components.

The evidence reported here suggests that the DP amino terminus does indeed contain sequences required for the association of DP with desmosomes. Ectopically expressed full-length tagged DP I apparently incorporated into desmosomes in some transiently transfected cells as demonstrated by the colocalization of tagged DP and endogenous Dsg in a punctate pattern at cell–cell borders by immunofluorescence. Deletion of 194 amino acids (18% of the amino terminus or 6.7% of the whole molecule) resulted in the loss of colocalization of tagged DP and Dsg in this characteristic punctate pattern at cell–cell interfaces. This observation suggests that sequences at the very amino terminus of DP either alone or in concert with other portions of the molecule are necessary for binding to an as yet unidentified binding partner(s) within the desmosome, thus allowing DP to integrate into the plaque. One might have predicted that the rod domain of DP may have been sufficient to integrate into desmosomes, as is the case with the sufficiency of the rod domain of keratin IF for incorporation into an established keratin IF network (Albers and Fuchs, 1989). However, this does not appear to be the case for DP, which apparently requires sequences in the amino terminal domain to target it to desmosomes. While the amino terminus of DP is required for incorporation, we cannot rule out the possibility that the rod domain may still play a crucial role in this process.

In addition to localizing to desmosomes in some cells, in other cells exogenous DP coaligned with and disrupted cytoplasmic IF. For any given transfected cell, tagged DP tended to either integrate into desmosomes or align with and disrupt IF. There are several, possibly interrelated, explanations for why not all cells transiently expressing exogenous full-length DP appear to incorporate it into desmosomes. The first, and simplest is that not all COS-7 cells make desmosomes with neighboring cells. Interaction along cytoplasmic IF networks might then occur by default. Secondly, in cells that do possess desmosomes but do not appear to incorporate tagged DP, cell surface binding sites for the amino terminus might be limited. Saturation of these sites could free DP to interact with the cytoplasmic IF. This limited availability of cell surface sites for DP as compared to the more abundant number of sites along IF may further inhibit incorporation. Interaction of DP with IF might then sequester these subunits such that they are no longer available for desmosomal incorporation. This idea is supported by the observation that DP.ΔC, which does not appear to interact with IF, can apparently incorporate more efficiently into desmosomes. A third possibility is related to the dynamic exchange of DP subunits into existing desmosomes. In contrast to the situation for IF networks, nothing is known about parameters governing exchange of DP into desmosomes. IF subunits are known to integrate rapidly into an existing IF network when introduced into cultured cells either by cDNA transfection (Albers and Fuchs, 1987 as an example) or microinjection of purified protein (Miller et al., 1991). This is probably due to the dynamic exchange of incorporated and unincorporated IF subunits (Vikstrom et al., 1992). In the case of cells transiently transfected with full length DP, slow exchange could contribute to the lack of incorporation into desmosomes.

Intriguingly, a proportion of transfected cells seem to efficiently incorporate ectopically expressed DP at cell surface sites. It is as yet unclear why this occurs in these cells. Possibly, heterogeneity among cells reflecting differences in physiological state and stage of the cell cycle could affect the ability of DP to incorporate into existing desmosomes or assemble into new desmosomes. We often observed that transfected cells with incorporated DP were apparently newly divided daughter cells. While desmosomes are not disrupted during mitosis (Baker and Garrod, 1993), assembly of new desmosomes at the newly formed cell–cell interface clearly must accompany cytokinesis. This could provide an opportunity for rapid incorporation of newly synthesized ectopically expressed DP I into cell junctions.

The complete lack of coalignment with and disruption of IF by the overexpression of DP.ΔC provides additional support for the idea that the carboxyl terminus of DP contains sites necessary and sufficient for interaction with IF. Because DP.ΔC can apparently incorporate into desmosomes, interaction with IF is probably not required for localization to desmosomes. While this result does not rule out the possibility that IF provide a route for DP to localize to desmosomes (Jones and Goldman, 1985; Pasdar and Nelson, 1988; Trevor and Steben, 1992), it suggests that other mechanisms can be used. Our results are consistent with previous suggestions that IF are not required for delivery of DP to desmosomes (Bologna et al., 1986; Mattey and Garrod, 1986; Duden and Franke, 1988). The existence of an IF independent pathway for delivery of DP to the cell surface could explain the apparently normal assembly of the desmosomal plaque in IF deficient simple epithelia (Baribault and Oshima, 1991) or in epithelia with disrupted IF (Vasser et al., 1991).

The Carboxyl Terminus of DP Interacts Differentially with Keratin and Vimentin IF Networks

Previously, we demonstrated that the carboxyl terminus of

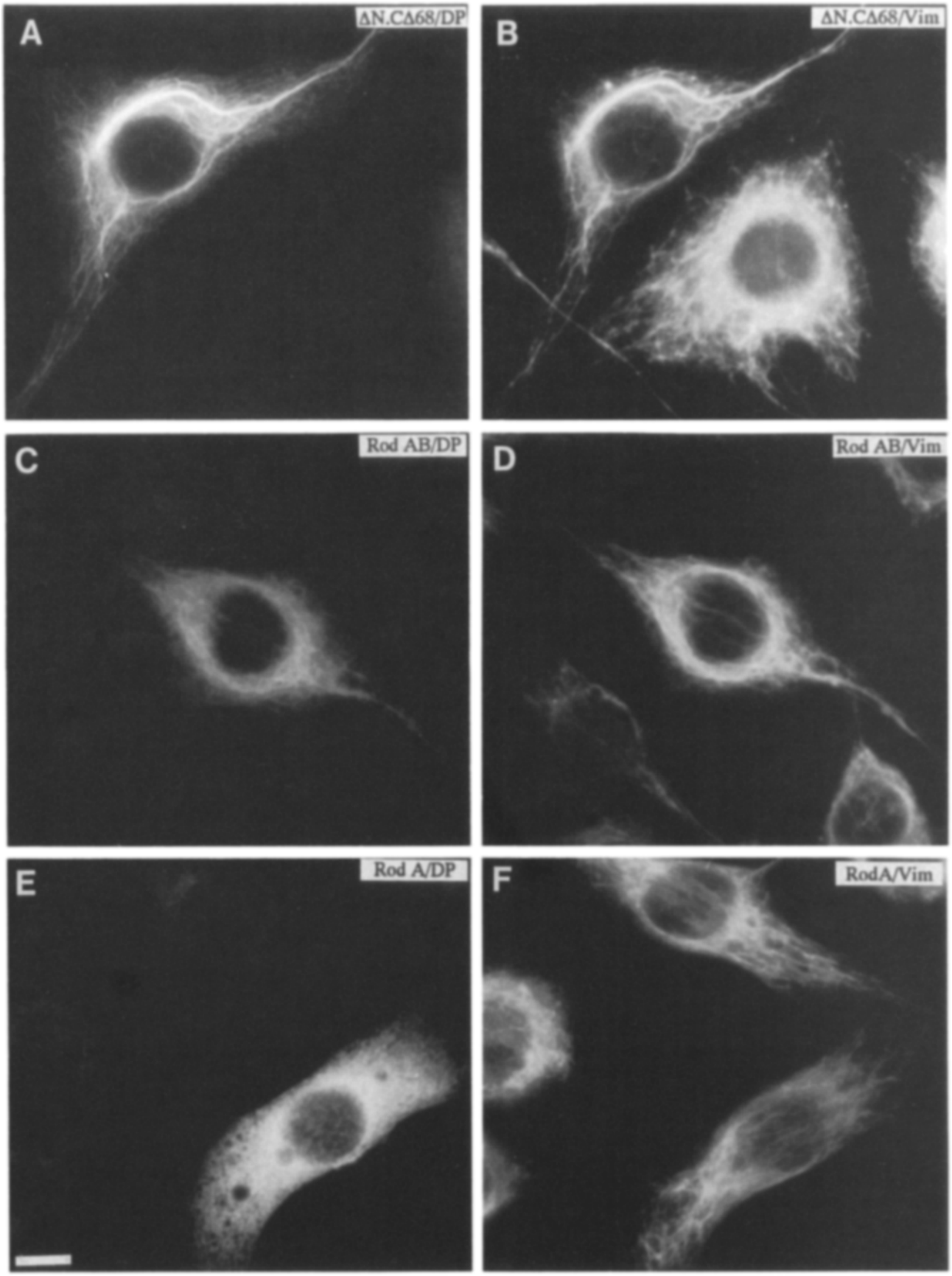


Figure 10. Indirect double label immunofluorescence of 3T3 cells transfected with pDP.ΔN.CΔ68 (*A* and *B*), pDP.RODAB (*C* and *D*), or pDP.RODA (*E* and *F*). 24 h after glycerol shock, *A*, *C*, and *E* were reacted with a mouse monoclonal to DP I and II (DP 2.15), and *B*, *D*, and *F* were reacted with a rabbit polyclonal to vimentin. Bar, 10 μm.

DP is necessary and sufficient for coalignment with and disruption of both keratin and vimentin IF networks (Stappenbeck and Green, 1992). These results did not rule out the possibility that accessory or linking proteins are required for this interaction. However, this seemed unlikely because if this were the case, any putative accessory proteins would have to be expressed at levels commensurate with that of the ectopically expressed DP (see for example the level of ectopic DP proteins relative to endogenous DP I; Fig. 6).

To further define the regions of the DP carboxyl terminus required for interaction with IF networks, DP.ΔN proteins with finer deletions within the carboxyl terminus were examined. This analysis led to the striking observation that DP.ΔN.CΔ68 was capable of coaligning with and disrupting vimentin IF but not keratin IF. This deletion of the 68 most carboxyl-terminal residues from DP.ΔN also led to a dramatic increase in its Triton extractability from transfected HeLa cells, which express keratin IF. Amino acids 48–68 residues from the carboxyl terminus were necessary for this interaction with keratin. Interestingly, this region shares some sequence similarity with both BPA and plectin, suggesting that this sequence could potentially be important for specific interactions with keratins for all three family members (Green et al., 1992). However, while the carboxyl-terminal 68 residues were necessary, they were apparently not sufficient for the coalignment with keratin IF. One possible explanation for the lack of coalignment with keratin IF by DP.ΔN.CΔ68 was that this deletion disrupted the conformation of an actual binding site for keratins located upstream from this sequence. However, this deletion must not completely disrupt the function of the remainder of the DP carboxyl terminus, as DP.ΔN.CΔ68 can still colocalize with vimentin. The 68 amino acid tail may also act in concert with another region of the carboxyl terminus of DP to form a binding site specific for keratins.

Because DP.ΔN.CΔ68, which contains the A, B, and C subdomains (Fig. 1), still coaligned with vimentin, we were able to test the importance of specific DP subdomains for interaction with vimentin IF by successively deleting them from DP.ΔN. Deletion of the C domain resulted in partial loss of coalignment with vimentin while deletion of the B and C subdomains resulted in complete loss of coalignment, suggesting that the B and C subdomains are required for the interaction. It is possible, however, that the specific subdomains present are not as important as their actual number. In fact, since the rod domain of DP.ΔN would presumably allow for dimerization (and possible higher order aggregation) a total of six subdomains (for both DP.ΔN and DP.ΔN.CΔ68) would presumably be available to interact with IF. Consistent with this model, the decrease in the number of subdomains available with DP.RODAB (four) and DP.RODA (two) correlated with decreased coalignment with vimentin (Fig. 1). In addition, DP.CT did not coalign with vimentin as well as DP.ΔN (Stappenbeck and Green, 1992). Since DP.CT presumably cannot dimerize, each molecule would have only three subdomains, and would be predicted to only partially coalign with vimentin IF. This model is supported by data obtained by overexpressing various domains of plectin in cultured cells (Wiche et al., 1993). In this case, four subdomains of the carboxyl terminus were required for colocalization with vimentin and keratin IF in transfected cells. However, it is still possible that a specific subdomain of either plectin or DP might be critical for this interaction.

Taken together, the deletion analysis presented here indicates that different elements of DP are important for interaction with keratin versus vimentin IF. For vimentin, increasing numbers of DP carboxyl-terminal subdomains apparently facilitates coalignment. Addition of the rod domain seems to further improve the interaction with vimentin IF, perhaps due to DP dimerization. In contrast, the rod domain is not required for interaction of DP with keratin IF, as the carboxyl terminus alone appears to be both necessary and sufficient (Stappenbeck and Green, 1992). Although the exact number of carboxyl-terminal subdomains required for DP interaction with keratin IF was not determined, one subdomain is apparently insufficient. The most striking difference between the two interactions, however, is that the 68 amino acids at the very carboxyl terminus of DP are required for interaction with keratin but not vimentin IF.

What might be the functional significance of the differential interactions of the DP carboxyl terminus with keratin versus vimentin IF? When both IF systems are present in the same cell, keratins have been reported to preferentially interact with desmosomes (Kartenbeck et al., 1984). Thus the keratin-specific sequences in DP may not only be necessary for specifying this interaction with DP, but may actually promote interaction with keratin over vimentin IF. This notion is supported by the resistance to Triton extraction of DP.ΔN in HeLa cells which contain keratin IF as compared to 3T3 cells which do not contain keratin IF networks. This differential interaction probably reflects structural differences between keratin versus vimentin IF. The globular end domains of keratin and vimentin IF are significantly different (Steinert and Roop, 1988); these differences may be responsible for differential interactions with DP. However, differences between the rod domains of vimentin and keratin must be considered as well. Although the rod domains of all IF are similar, the 1B and 2B portions of the keratin and vimentin rods cannot be interchanged without affecting their function (McCormick et al., 1991). Thus, the rod domains of both IF types could be the primary sites of interaction with DP as previously predicted (Green et al., 1990) yet still provide specificity.

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