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Antibodies Differentiate Desmosome-Form and Nucleus-Form Pinin: Evidence That Pinin Is a Moonlighting Protein with Dual Location at the Desmosome and within the Nucleus

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Pinin is a desmosome-associated protein occurring in epithelia, cardiac muscle, and meninges. This molecule was found to be capable of enhancing cell junction formation and thought to play a key role in reorganization and stabilization of the desmosome-intermediate filament complex in epithelial cells (*J. Cell Biol.* (1996) 135, 1027–1042). Recently a protein, claimed to be localized exclusively in the nucleus, however, with amino acid sequence identical to pinin, was reported (*E. J. Cell Biol.* (1998) 75, 295–298). Here I present evidence that pinin exists simultaneously at the desmosome and within the nucleus by generating location-specific monoclonal antibodies. Although the desmosome-form (d-form) and the nucleus-form (n-form) pinin share identical amino acid sequences as demonstrated by cDNA library screening and DNA sequencing, they exhibit remarkably different biochemical properties, reflecting the apparent different multiprotein nature of their differential cellular locations. In addition, the d-form pinin is characterized by a dynamic transport process which involves the gradual diminishing of nuclear materials relative to enhanced anchoring of pinin to the desmosome upon mature cells. Finally I demonstrate that pinin exists in two forms of different gene product: pinin1 and pinin2. These data argue strongly against the statement that pinin is an exclusive nuclear protein and support the notion that pinin is a moonlighting protein with more than one function as a consequence of its dual cellular location.

Key Words: **desmosome; nucleus; pinin; antibody generation.**

Abbreviations used: DRS, domain rich in serine; RNP, ribonucleoprotein; PBS, phosphate buffer saline; MDCK, Mardin Darby Canine Kidney; d-form, desmosome-form; n-form; nucleus-form; SDS, sodium dodesylsulfate; PAGE, polyacrylamide gel electrophoresis.

Desmosomes are punctate adhesive intercellular junctions associated with cytoplasmic intermediate filament network in epithelia, cardiac muscle, meninges and follicular dentritic cells (1). The major molecular components of desmosomes have been well characterized in the past couple of years. These include transmembrane glycoprtoeins desmoglein and desmocollin, both are members of cadherin families of calcium-dependent cell adhesion molecules, and plaque-associated proteins which comprise desmoplakin, plakoglobin and plakophilin (1, 2). In addition to the proteins constitutive to the desmosome, accessory proteins, such as desmocalmin and desmoyokin, have also been reported to exist in a subset of tissues containing desmosomes (3, 4), their definite roles with regard to desmosome functions and structures, however, are not yet established.

We have previously identified a novel desmosome-associated molecule, which was found to localize to the cytoplasmic face of the desmosomal plaque in the convergence of intermediate filaments and whose occurrence in the desmosome seems to relate to the ordered organization of cytoplasmic intermediate filament network (5). This molecule was therefore named pinin, pin intermediate filaments down. Functional studies indicated that pinin can induce junction formation and enhance cell aggregation following transfection full-length pinin cDNA *in vitro,* suggesting that pinin play a role in relation to cell-cell adhesion (6). However, a protein, termed "domain rich in serine" (DRS), with amino acid sequences almost identical (>95%) to pinin and claimed to be localized exclusively within the nuclei of all tissues and cell types with/without desmosomes, was reported recently in the literature in which it was considered to be the ribonucleoprotein (RNP) structure of the speckle category colocalized with certain splicing factors and Sm-proteins (7). In order to solve the apparent discrepancy between pinin and DRS

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in cellular locations and functions, I generated monospecific antibodies based on a stretch of 36 amino acids common to the extreme carboxyl-end of both proteins. The results of this study indicated that pinin is not only associated with the desmosome but also exists within the nucleus and it displayed remarkably different biochemical properties in different location. In addition I provide evidence that pinin exists in two forms of different gene product, pinin1 and pinin2. The data also argue strongly against the statement that the pinin/DRS protein occurs exclusively as a component of RNP structure in the nucleus.

MATERIALS AND METHODS

Reagents. Dulbeco's modified Eagle medium (DMEM), Hank's medium RPMI 140 medium and fetal calf serum (FCS) were purchased from Gibco BRL (Gaithersburg, MD). Phenylmethylsulfonyl fluoride (PMSF), chemostatin, leupeptin, and pepstatin were purchased from Sigma Chemical Co. (St. Louis, MO). Glutathione Sepharose 4B was purchased from Pharmacia Biotech Co. (Uppsala, Sweden). All molecular biology reagents, including restriction enzymes and *Taq* polymerase, were purchased from New England Biolab (Beverly, MA).

Cell culture. MDCK cell line of passages 10–60 were maintained in DMEM and supplemented with 10% FCS, 2 mM glutamine and 200 U/ml each of streptomycin and penicillin G. Cells were passed with 0.1% trypsin and 0.04% EDTA in Hank's meidum.

Expression and purification of recombinant proteins. FP08 was produced by ligation the EcoRI-XhoI fragment of pinin cDNA ss-13 to the pGEX4T3 vector (Pharmacia Biotech Co., Uppsala, Sweden) and expressed in DH5α cells. Preparation of peptide 3a as well as purification of recombinant proteins were performed as previously described (6). In brief, bacteria that were successfully transformed with recombinant vector were induced with 0.1 mM IPTG in LB for 3 h at 37°C. Purifications of expressed proteins were accomplished by absorption of fusion proteins form bacterial cell lysate to glutathione sepharose 4B followed by elution with 5 mM glutathione in 50 mM Tris-HCl, pH 8.0.

Antibodies production. Fusion protein, containing peptide 3a, was used as immunogen for polyclonal antibody production in rabbits. The initial inoculation, containing 300 μg of fusion protein, and four boost injections of 120 μg was given at three-week intervals. Two weeks later, final boost rabbit serum was harvested. Western blot and immunofluorescence analysis were carried out with the reactive serum and pre-immune serum. For monoclonal antibody production, FP08-containing fusion protein was used as immunogen and antibody generation was performed as described previously (5). In brief, 20 μg FP08 was mixed with complete Freunds adjuvant to inject Balb/C mice. Subsequent to three or four boost injections, at 2-week intervals, mice were sacrificed and splenocyte was fused with NS1 myeloma cells. Hybridoma cells grown in HAT-containing RPMI medium were plated into 24-well dishes. Supernatants were screened by immunofluorescence microscopy of MDCK cells cultured on coverslip and Western blot using whole cell protein extracts. Cells of positive wells were selected and cloned by limited dilution.

Screening of cDNA library. An oriented MDCK cDNA library constructed in UNI-ZAP XR vector (Stratagene, La Jolla, CA) was prepared according to the manufacturer's instruction. A total of 1,000,000 phage plaques grown initially at 42°C for 5–6 h were replicate plated to nitrocellulose filters which were prepared for screening with poly3a or 08YD6 after additional 3.5 h incubation at 37°C. The immuno-screening procedures were essentially the same as those for Western blot described below. Positive clones were plaque-purified and subjected to second round of immuno-screening followed by in-vivo excision of the pBS-SK phagemids which were used for subsequent restriction mapping and DNA sequencing.

Immunofluorescence microscopy. MDCK cells cultured on coverslip for either 16 h or 2 days were treated with the following different protocols; the first set was permeabilized with acetone for 3 min at −20°C, the second set was in addition processed for 30 min with 0.5% Triton X-100 in PBS following acetone permeation. Cell cultures were then washed briefly in PBS and incubated with various antibodies for 1 h at room temperature. The antibodies used were FP08B1, 08YD6, both are monoclonal antibodies generated against FP08, and poly3a, polyclonal serum directed against peptide 3a. For double label study, a polyclonal serum against desmoplakin (Serotec Co., Oxford, England) was used at 1:200 dilution. After three 5 min washes in PBS, the samples were incubated with FITC-conjugated goat anti-mouse or goat anti-rabbit IgG (Jackson Laboratory, West Grove, PA) for 1 h at room temperature. Tex-red conjugated goat anti-rabbit IgG was used for double label immunofluorescence. The samples were then washed extensively with PBS, mounted with 50% glycerol containing 0.4% n-propylgallate and examined with a photo microscope (Carl Zeiss, Germany) equipped with epifluorescence.

Western blot. MDCK cell lysate were prepared by lysing cell culture in RIPA buffer (50 mM Tris, pH 8.0, 0.14 M NaCl, 0.2% SDS, 1% Triton X-100 and 0.5% sodium deoxycholate) containing 1 mM PMSF and 1 mg/ml each of pepstatin, leupeptin and chemostatin. Samples were sonicated for 30 s on ice, stored at −70°C and boiled for 5 min prior to electrophoresis on a 7.5% polyacrylamide gel. Western blot was then performed as described previously (19). Proteins were transferred from the gel to nitrocellulose paper. The paper was blocked with 5% non-fat dried milk in PBS followed by washing in PBS. Primary antibodies incubations, with antibodies listed above, were carried out for 1 h at room temperature. After extensive wash in PBS, the paper was incubated for 1 h with HRP conjugated goat anti-mouse or anti-rabbit IgG (diluted 1:1000 in PBS, Jackson Laboratory, West Grove, PA). The peroxidase-labeled blots were reacted with 0.5 mg/ml diaminobenzidine and color reaction were developed using 0.01% hydrogen peroxide.

Genomic PCR and reverse transcriptase-PCR (RT-PCR). MDCK cultured in 100-mm dishes were used for genomic DNA and total RNA preparation. Genomic DNA was extracted using DNAzol (Molecule Research Center, Inc., Cincinnati, OH) as described by the manufacturer's instruction. Total RNA was extracted with Trireagent (Molecule Research Center, Inc., Cincinnati, OH) according to the protocol provided by the manufacture. CDNA synthesis was performed with 2 μg total RNA primed with a 17 mer oligo-dT using 100 U superscript II reverse transcriptase (Gibco BRL, Gaithersburg, MD) at 42°C for 60 min. PCR was carried out with a primer set spanning portion of the exon 9 of MDCK pinin gene using 2 μl synthesized cDNA or 100 ng genomic DNA as templates. The conditions for PCR is 35 cycles each of 94°C for 30 s, 55°C for 40 s and 72°C for 40 s. The nucleotide sequences for PCR are as follows; gsp10 (sense): 5′-aagcagcaggatagtcaacc-3′; gsp02 (antisense): 5′-ttaacgccttttgtctttcc-3′. Subsequent to PCR the products were subcloned directly into pCRII-Topo T-vector (Invitrogen, Carlsbad, CA) for restriction confirmation and sequence analysis.

RESULTS AND DISCUSSION

Dual Location of Pinin within the Nucleus and at the Desmosome

In our initial search of pinin's function and subcellular location, we found pinin was localized preferentially to the cell-cell contact area, predominantly the desmosome of intercellular adherens junction (5). However, obvious nuclear staining can be observed when epitope-tagged full-length pinin was transfected into

cells cultured *in vitro* (unpublished data), and this nuclear distribution of pinin can not be detected by the original monoclonal antibody, 08L, which was previously used to identify the desmosomal location of pinin and isolate pinin protein (6). These results raised an interesting question whether pinin was shuttled between two cellular compartments, the nucleus and the desmosome, or pinin resided both within the nucleus and at the desmosome. If pinin indeed exists within the nucleus, they may take on a conformation different from those occurring at the desmosome or for some reasons 08L antibody epitopes are masked, thereby preventing our observation of pinin within the nucleus. To solve the apparent subcellular different location of pinin, I adopted a strategy by making mono-specific antibodies against a stretch of amino acids located in the carboxyl-end of pinin (Fig. 1A). This stretch of sequence contained 36 amino acids rich in Ser-Arg and Arg-Ser dipeptide, motifs frequently found in splicing factors or splicing-associated proteins (8, 9). Theoretically antibodies against this peptide should be reactive predominantly with nuclear proteins due to the presence of sequences frequently occurring in RNA-binding proteins, identification of antibodies reactive both with desmosomal pinin and nuclear pinin would therefore support pinin's dual cellular location.

In addition to FP08, which harbors the carboxyl-end 36 aa, I prepared as immunogen peptide 3a (Fig. 1A) fused to glutathione S-transferase. 3a comprise mostly the PQLQ domain of pinin and is 100 aa in length. Following recombinant protein expression and purification (Fig. 1B), FP08 was used to generate monoclonal antibodies while 3a used for polyclonal antibodies generation. Two monlclonal antiboides, FP08B1 and 08YD6, and one polyclonal antibody, poly3a, were developed after several rounds of selection and clarification. Immunofluorescent studies of cultured canine kidney epithelial cell line MDCK indicated that FP08B1 stained predominantly areas of cell-cell contacts (Fig. 2A) in a manner reminiscent of the staining pattern of the original pinin antibody, 08L (5). Careful examination of the distribution patterns of FP08B1 revealed that the immunogens were arranged as dots along the cell contact areas (Fig. 2A, arrows) which were colocalizaed with those of desmoplakin staining (Fig. 2B, arrows), a hall mark of desmosomal proteins. On a stark contrary, 08YD6, another monoclonal antibody derived from the same FP08 antigen as used for generation of FP08B1, immunostained predominantly MDCK cell nucleis in granular forms throughout the nucleoplasm (Fig. 2D), whereas nucleoli and chromosomes were devoid of staining. Similar nuclear staining patterns were observed for poly3a polyclonal antibodies (Fig. 2C). Detailed studies of 08YD6 and poly3a immunostaining patterns indicated that their antigens behaved like typical karyophilic proteins during mitosis; they are released upon prometaphasic disruption of the nuclear envelop in a finely dispersed form over the cytoplasm (Figs. 2C and 2D, arrowheads) but rapidly re-accumulate during telophase in the forming daughter nuclei. From the immunofluorescent staining point of view, 08YD6 and poly3a antigen acted exactly like the DRS (domain rich in serine) protein described by Brandner *et al.* (7), which was located exclusively within the nucleus but was identical to pinin in amino acid sequence.

The major question was then whether 08YD6 and poly3a antigens were pinin, though they presented a total different cellular location from pinin. Screening of a MDCK λZAP oriented cDNA library was performed with 08YD6 and poly3a and a full-length clone poly3a(5) was identified and characterized. Sequence analysis of poly3a(5) revealed that it was almost identical to those of pinin [Cpinin1(n-form) vs. Cpinin1(d-form), Fig. 4C] with only six synonymous substitutions out of 769 amino acids. The identity of 08YD6 antigen to pinin was further supported by Western blot analysis, in which 08YD6, poly3a and FP08YD6 recognized a protein with identical molecular weight of ~140 kDa in MDCK cell lysate (Fig. 1C, arrow), suggesting pinin simultaneously occur within the nucleus and at the desmosome. These data therefore strongly speaks against the statement of Brandner *et al.* (7) who concluded that pinin/DRS was an exclusive nuclear protein not associated with desmosomes. The molecular basis for the contradictory reports on the immunolocalization of the pinin/DRS protein may be the result of epitope spreading caused by changes in conformation and antibody accessibility in the different multiprotein complex containing pinin/DRS. Generation and selection of multiple mono-specific antibodies is therefore a better resolution to identify multiple protein conformations than is solely production of polyclonal antibodies.

Desmosome- and Nucleus-Form Pinin Exhibited Different Biochemical Properties

According to the results presented above, it's obvious that two different cellular locations of pinin should not be sequence-dependent, such as generated by the alternative splicing of the message, rather they may be independent to each other or caused by differential physiological state. To characterize the physiological relation of desmosome-form (d-form) and nucleus-form (n-form) pinin, I carried out a series of experiments employing immunofluorescent staining in combination with different fixation and extraction protocols. The nuclear location of pinin was not significantly different throughout the culture period and under detergent treatment but was a ubiquitous and constitutive feature seen with the 08YD6 monoclonal antibody (Figs. 3E and 3F). FP08YB1, however, displayed readily differentiable immunostaining patterns in 16 h and 2

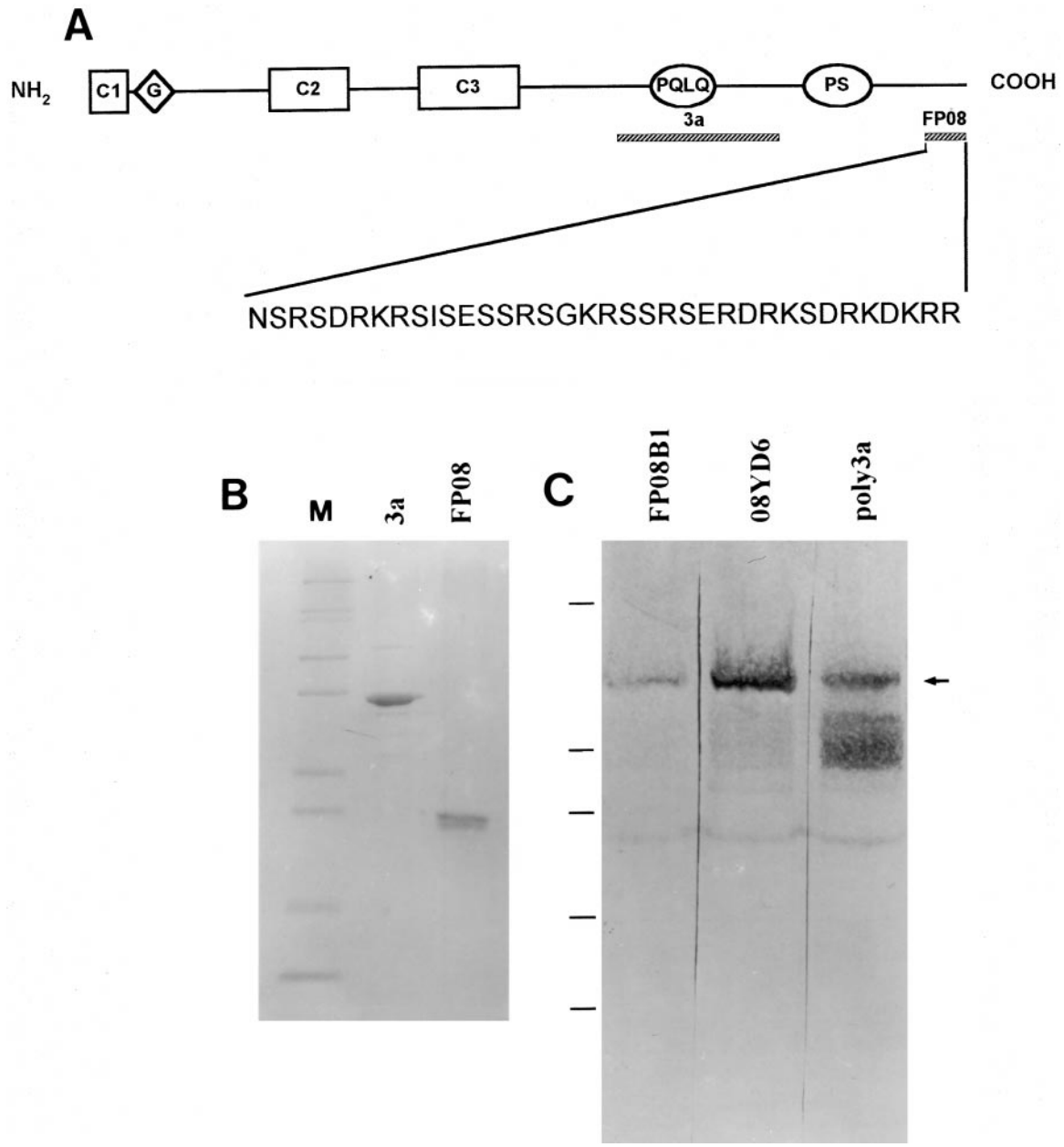

FIG. 1. (A) A schematic diagram showing the domain structure of canine pinin and the location of the immunogen (3a and FP08) used to generate antibodies. The amino acid sequences of FP08, which contains 36 amino acids and is situated at the carboxyl-terminus, are displayed in one letter code. C1, C2, and C3: coiled-coil domain; G; glycine loop; PQLQ: glutamine-proline domain; PS: polyserine domain. (B) SDS-PAGE profiles of the purified recombinant protein containing peptide 3a or FP08. M represents molecular weight standard in the decreasing order of 200, 116, 97, 66, 55, 36, 31, 21, and 14 kDa, respectively. (C) Western blot analysis of MDCK cell lysate reacting with various antibodies generated against 3a (poly3a) or FP08 (FP08B1 and 08YD6). All three antibodies recognize a single molecular species with molecular size of ~140 kDa (arrow). The horizontal lines on the left margin represent protein standards.

days MDCK culture; the former was stained not only cell-cell contact areas but also fine granules inside nuclei (Fig. 3A, arrows), the latter was stained predominantly desmosomes (Fig. 3C). Extraction with 0.5% Triton, however, led to diminishing of cell peripheral pinin staining in younger MDCK culture, leaving behind only intranuclear granular staining (Fig. 3B). Contrary to the younger culture, the older MDCK cells retained pinin immunostaining at cell peripheries even after prolonged detergent extraction (Fig. 3D).

The above data pinpointed that the nuclear and desmosomal location of pinin seems to be an independent event relative to each other. Besides, both forms showed remarkably different biochemical properties; n-form is structure-bound and not detergent extractable, whereas the extractability of the d-form is depen-

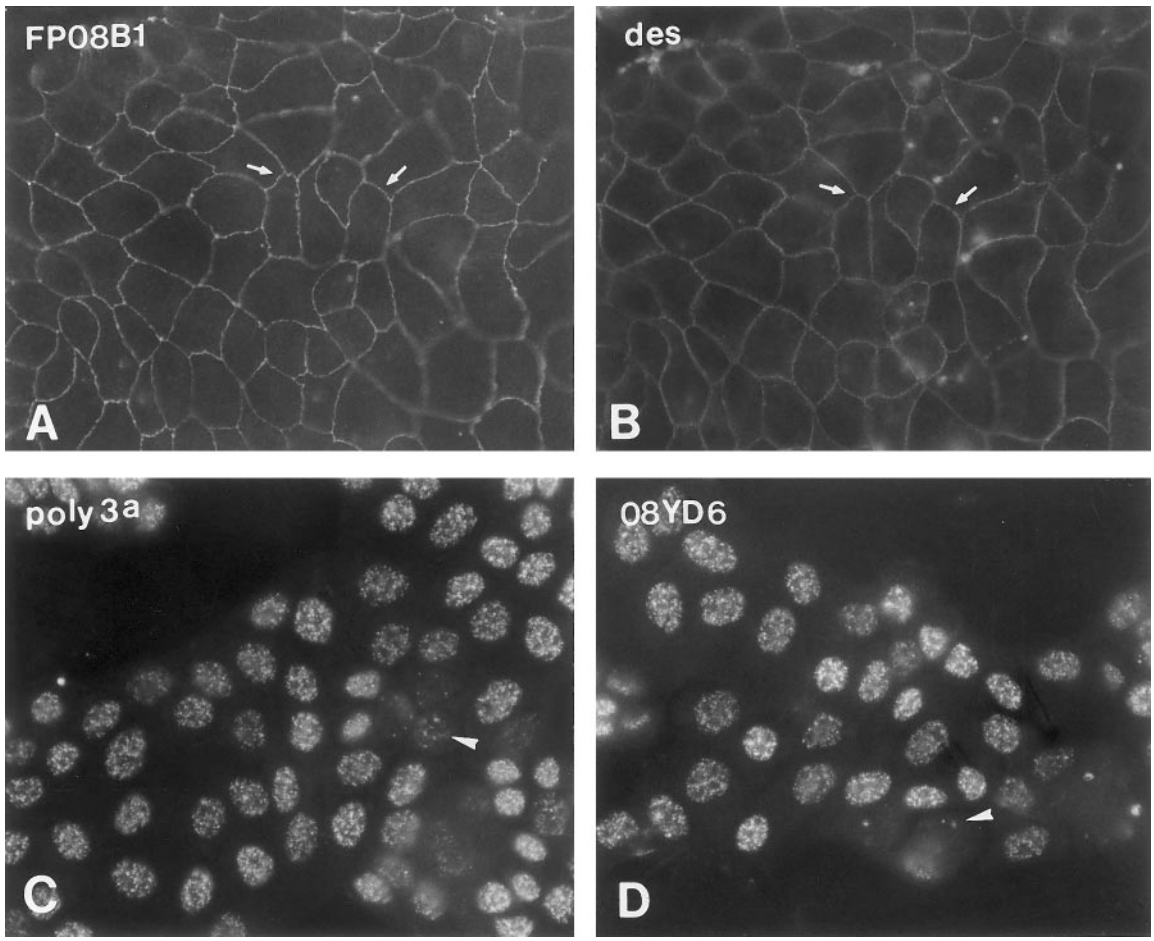

FIG. 2. Immunofluorescence micrographs of mature MDCK cells labeled with antibodies against 3a (poly3a) and FP08 (FP08B1 & 08YD6). FP08B1 (A) stained predominantly cell-cell contact areas with a punctate appearance (arrows) which can be seen colocalized with those of desmoplakin staining (B, arrows). Poly3a (C) and 08YD6 (D) stained instead predominantly cell nuclei with reaction products distributed in granular forms throughout the nucleoplasm. Note the dispersion of immunostained materials to cytoplasm during mitosis (arrowheads in C and D).

dent on the extent to which cells mature, with increasing pinin binding to desmosomes or/and intermediate filaments as cells age. In addition, the dynamic appearance of d-from pinin within the nucleus in only younger culture and increasingly robust pinin staining at cell peripheries of older culture suggested a gradual translocaton of FP08B1-positive material from intranuclear granulars to cell boundaries upon cells matures. A potential nuclear pool seems to occur to support the continuing maturation of d-form pinin. It's not uncommon to find pinin exists in both nuclei and desmosomes. Other junction-associated proteins, for examples, symplekin (10), a novel tight junction-associated protein, and plakophilin (11, 12), a desmosomal plaque protein, occur simultaneously in both nuclei and cell junctions. Desmoyokin, another desmosome-associated protein, has been demonstrated to translocate from nuclei to cell peripheries in keratinocytes in a protein kinase C-dependent manner (13). Although the exact nature of the nuclear pool of d-form pinin remains elusive, it would be interesting to unravel its difference from the n-form pinin and the regulatory mechanism that harnesses the nuclear export of d-form pinin.

Pinin Exists in Two Forms of Different Gene Products

Initially I considered that alternative splicing might contribute to the differential location of pinin within the nucleus and at the desmosome. In the course of looking for such product, I coincidentally identified a second pinin gene product, which was named pinin2 as opposed to the original one, pinin1 (Fig. 4B). Using a pair of primers, gsp10 and gsp02, which spans exon 9 of MDCK gene (Fig. 4A), I resolved two messages 138bp apart upon RT-RCP of MDCK total RNA (Fig. 4B, lane 2). The upper band was at a level equal to that of PCR products derived from both the original pinin cDNA-ss13 (Fig. 4B, lane 3) and a λ genomic clone (DG#7) containing pinin1 exons (Fig.4B, lane 5), respectively. The lower band was found also in products of MDCK

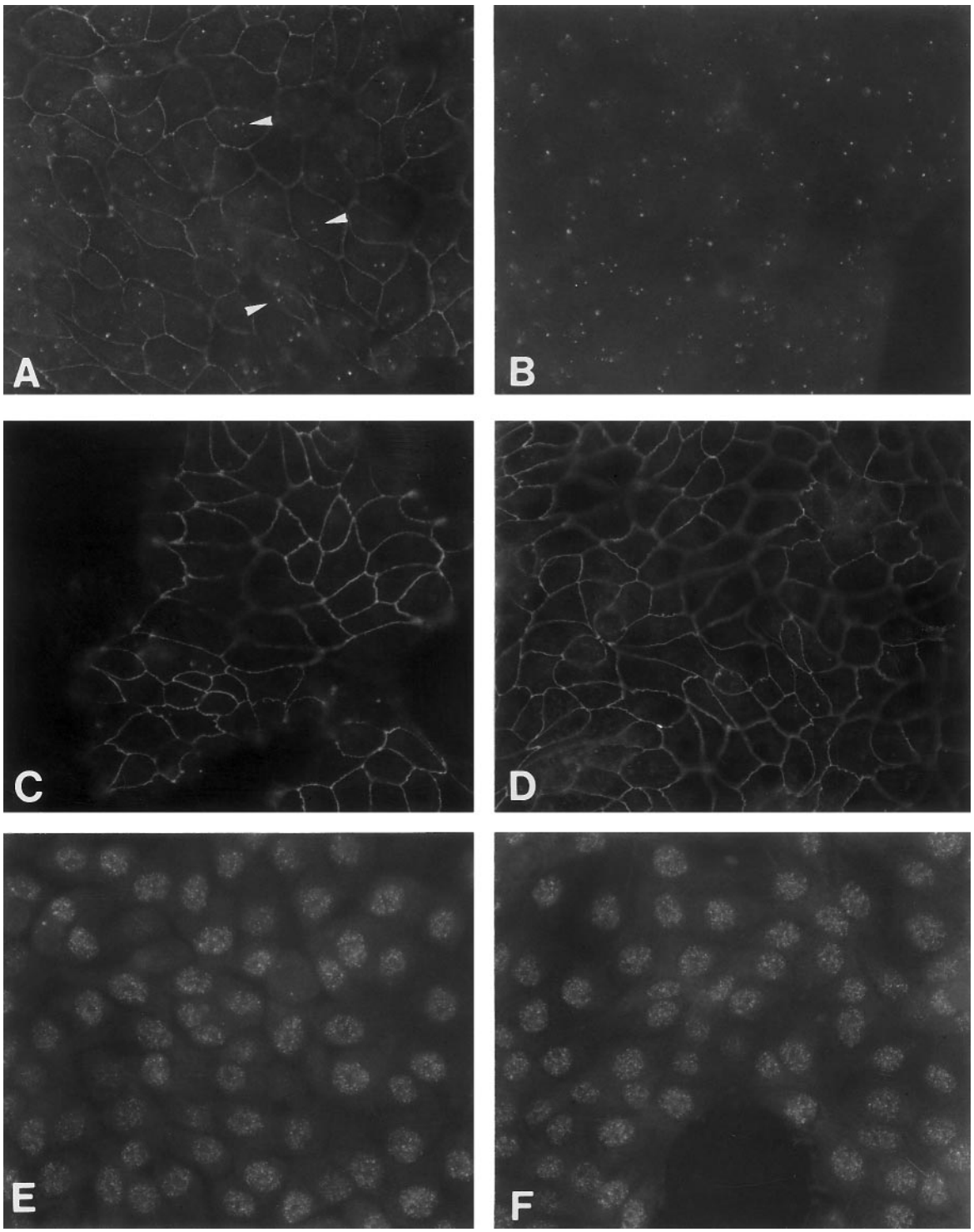

FIG. 3. Immunofluorescence micrographs demonstrating the maturation-dependent translocation of desmosome-form (d-form) pinin from the nucleus to the cell periphery. MDCK cultured for 16 h (A and B) or 2 days (C and D) are processed for immunostaining with FP08B1 following acetone treatment (A and C) or acetone plus Triton extraction (B and D). Younger MDCK culture demonstrates staining at both the cell periphery and within the nucleus (A, arrows), whereas the cell peripheral staining is washed away during detergent extraction (B). Mature MDCK cells are stained predominantly at cell peripheries with little or no nuclear staining (C). Detergent treatment does not alter the staining pattern (D). The nuclear distribution of 08YD6 staining is not affected by either the degree of cell maturation (E, 2 day culture) or detergent extraction (F, 2 day culture).

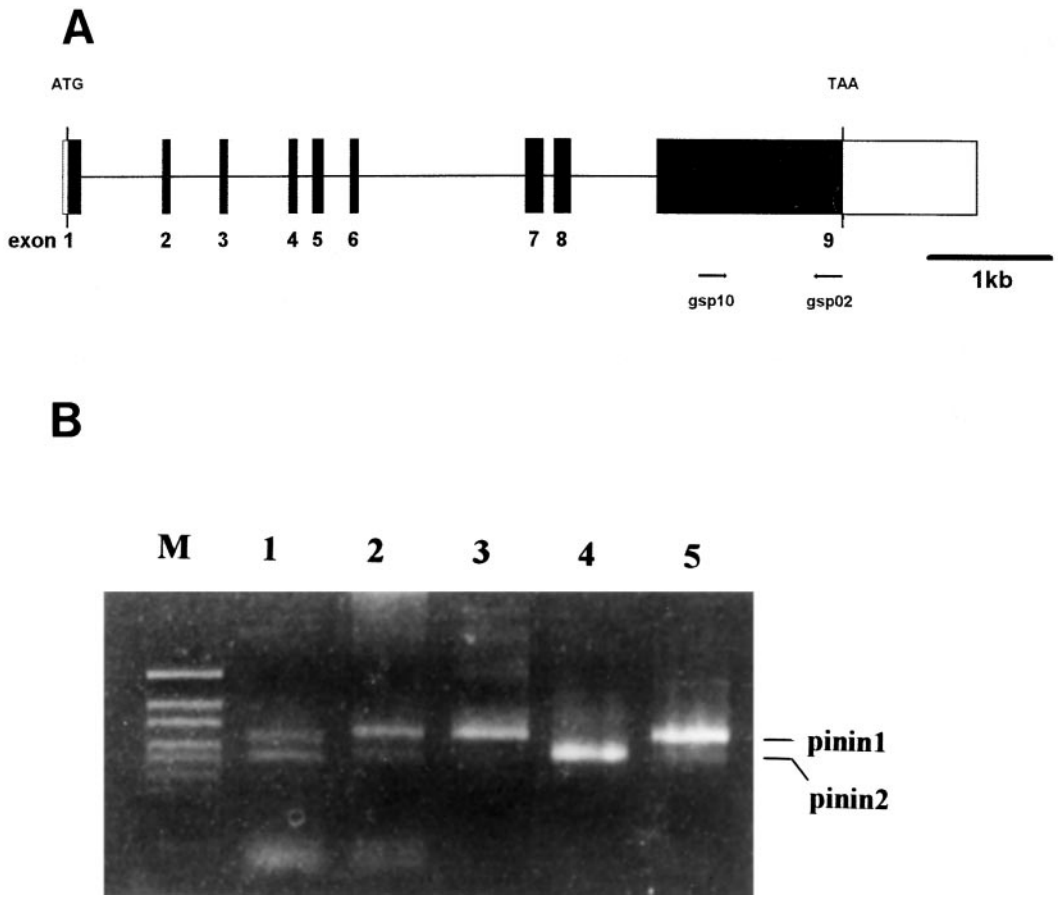

FIG. 4. (A) A schematic showing the genomic structure of the canine pinin and position of the primer pair, gsp10 and gsp02. The thin line represents intron while rectangles are exons which can be divided into untranslated region (white boxes) and open reading frame (black boxes). (B) Agarose gel electrophoresis demonstrating pinin existing in two forms of different gene products. PCR was carried out with MDCK genomic DNA (lane 1), total RNA (lane 2), the original pinin cDNA ss-13 (lane 3), genomic λ clone DG#3 (lane 4), or genomic λ clone DG#7 (lane 5) as well as with gsp10 and gsp02. The upper band (pinin1) showed sequence identity to the previously described pinin, whereas the lower band (pinin2) shared partial sequence homology to the original pinin cDNA. (C) Comparison of deduced amino acid sequences of canine desmosome-form pinin (Cpinin1 d-form), nucleus-form pinin (Cpinin1 n-form) and a second canine pinin gene products (Cpinin2). Residues that are underscored with a dot represent conservative substitution. Note the apparent difference in PQLQ domain.

genomic PCR and PCR product of a second λ genomic clone (DG#3, data unpublished), suggesting pinin exist in a form different from the original gene product. Comparison of DNA sequences and deduced amino acids of pinin2 with pinin1 (Fig. 4C) further supported the view that pinin2 was derived from a second gene product. This finding is consistent with that of Brandner *et al.* (7) who reported that DRS of Xenopus occurs in two forms of different gene products. As to the function and cellular location of pinin2 as well as whether pinin2 is involved in the differential localization of pinin are currently under study.

Based on the fact that a single pinin gene product, pinin1, occurs simultaneously in obviously different cellular compartment, within the nucleus and at the desmosome, and performs functions unrelated to each, I may declare pinin as a moonlighting protein. The function of a moonlighting protein can vary as a consequence of changes in cellular location, cell type or the cellular concentration of a ligand, substrate (14). For example, many lens crystallins and some ribosomal proteins are identical to cytoplasmic proteins and were apparently recruited during the evolution of the eye and the ribosome, respectively (15, 16). Since pinin has been suggested to function as a mediator of multiprotein complexes (17, 18) in various cellular locations, I consider that the interaction of pinin with different polypeptides to form different multisubunit complexes can result in pinin's switch in function. This theme is supported by our two-hybrid experiment in which we found some pinin-interacting proteins that either are nuclear in nature or are apparently epithelium-specific (manuscript in preparation).

To summarize, I present evidence that pinin/DRS protein occurs simultaneously within the nucleus and at the desmosome and both forms of pinin are actually the same gene product of *Pinin1,* which shares partial sequence homology to a second pinin gene, *Pinin2.* I

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Cpinin1(d-form)  MAVAVRTLQEQLEKAKESLKNVDENIRKLTGRDPNDVRPIQARLLALSGP    50
Cpinin1(n-form)  MAVAVRTLQEQLEKAKESLKNVDENIRKLTGRDPNDVRPIQARLLALSGP    50

Cpinin1(d-form)  GGGRGRGSLLLRRGFSDSGGGPPAKQRDLEGAVSRLGGERRTRRESRQES   100
Cpinin1(n-form)  GGGRGRGSLLLRRGFSDSGGGPPAKQRDLEGAVSRLGGERRTRRESRQES   100

Cpinin1(d-form)  DPEDDDVKKPALQSSVVATSKERTRRDLIQDQNMDEKGKQRNRRIFGLLM   150
Cpinin1(n-form)  DPEDDDVKKPALQSSVVATSKERTRRDLIQDQNMDEKGKQRNRRIFGLLM   150

Cpinin1(d-form)  GTLQKFKQESTVATERQKRRQEIEQKLEVQAEEERKQVENERRELFEERR   200
Cpinin1(n-form)  GTLQKFKQESTVATERQKRRQEIEQKLEVQAEEERKQVENERRELFEERR   200

Cpinin1(d-form)  AKQTELRLLEQKVELAQLQEEWNEHNAKIIKYIRTKTKPHLFYILGRMCP   250
Cpinin1(n-form)  AKQTELRLLEQKVELAQLQEEWNEHNAKIIKYIRTKTKPHLFYIPGRMCP   250

Cpinin1(d-form)  ATQKLIEESQRKMNALFEGRRIEFAEQINKMEARPRRQSMKEKEHQVVRN   300
Cpinin1(n-form)  ATQKLIEESQRKMNALFEGRRIEFAEQINKMEARPRRQSMKEKEHQVVRN   300

Cpinin1(d-form)  EEQKAEQEEGKVAQREEELEETGNQHNDVEIEEAGEEEEKEIGIVHSDAE   350
Cpinin1(n-form)  EEQKAEQEEGKVAQREEELEETGNQHNDVEIEEAGEEEEKEIGIVHSDAE   350

Cpinin1(d-form)  KEQEEEEQKQEMEVKIEEETEVRESEKQQDSQPEEVMDVLEMLLHVAVKN   400
Cpinin1(n-form)  KEQEEEEQKQEMEVKMEEETEVRESEKQQDSQPEEVMDVLEMV--ESVKN   398
Cpinin2                                  KQQDSQPEEVMDVLEMV--ESVKH    22

Cpinin1(d-form)  VIAEQEVMETNQVESVEPSENETSKELEPEMEFEVEPDKECKSLSPVREN   450
Cpinin1(n-form)  VIAEQEVMETNQVESVEPSENETSKELEPEMEFEVEPDKECKSLSPVREN   448
Cpinin2          VIAEQEVMETNQVESIEPSENETSKELEPEMEFDVEPDKECKSLSPGKEN    72

Cpinin1(d-form)  ASALEMENEPEEKEERESEPQPEPVRHLQPLPQPEPEPELQPEPQPQLQP   500
Cpinin1(n-form)  ASALEMENEPEEKEERESEPQPEPVRHLQPLPQPEPEPELQPEPQPQLQP   498
Cpinin2          INSQEVEKESEEKEEKEEKE-----------PEPQPEPVAQPQPPPQPLP   111

Cpinin1(d-form)  EPQLQPQLQLQLQPQPQSQSQPQPQLQLPLPLPLQPQPQVQAQSQPQAVL   550
Cpinin1(n-form)  EPQLQPQLQL--QPQPQSQSQPQPQLQLPLPLPLQPQPQVQAQSQPQAVL   546
Cpinin2          ----------------------------------QSQPHSQPHSQPQPVL   127

Cpinin1(d-form)  QPQPVSQPETLPLAVLQAPVQVIQEQGHLLPERKEFPVESVKLTEVTVEP   600
Cpinin1(n-form)  QPQPVSQPETLPLAVLQAPVQVIQEQGHLLPERKEFPVESVKLTEVTVEP   596
Cpinin2          QSQPLCQPETLPLAVLQPPPQVIQEQGNLLPERKDFPLESIKLPEVSVEP   177

Cpinin1(d-form)  VLIVHSDSKTKTKTRSRSRGRARNKTSKSRSRSSSSSSSSSSSTSSSSGG   650
Cpinin1(n-form)  VLIVHSDSKTKTKTRSRSRGRARNKTSKSRSRSSSSSSSSSSSTSSSSGS   646
Cpinin2          VLTVHSENKSKNKTRSRSRGRARNKTSKSRSRSSSSSSSSSSSTSSSSGS   227

Cpinin1(d-form)  SSSSGSSSSRSSSSSSSSTSGSSRRDSSSSTTSSSESRSRSRGRGHNRDR   700
Cpinin1(n-form)  SSSSGSSSSRSSSSSSSSTSGSSRRDSSSSSISSSESRSRSRGRGHNRDR   696
Cpinin2          SSSSGSSSSRSSSSSSSSTSGSSSRDSSSSTSSSSESRSRSRGRGHNRDR   277

Cpinin1(d-form)  KHRRSVDRKRRDTSGLERSHKSSKGGSSRDTKGSKDKNSRSDRKRSISES   750
Cpinin1(n-form)  KHRRSVDRKRRDTSGLERSHKSSKGGSSRDTKGSKDKNSRSDRKRSISES   746
Cpinin2          KHRRSVDRKRRDTSGLERSHKSSKGGSSRDTKGSKDKSSRPDRKRSISES   327

Cpinin1(d-form)  SRSGKRSSRSERDRKSDRKDKRR   773
Cpinin1(n-form)  SRSGKRSSRSERDRKSDRKDKRR   769
Cpinin2          SRSGKRSSRSERDRKSDRKDKRR   350
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FIG. 4—*Continued*

also showed that the d-form and n-form pinin each displayed remarkably different biochemical properties and translocation of d-form to its destiny location is dynamic regulated by a nuclear pool of unknown nature. Experiments are now undergoing to resolve the functional significance of pinin in the context of regulated transport occurring between desmosomes and nuclei.

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