Identification of a second protein product of the gene encoding a human epidermal autoantigen

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A 230 kDa polypeptide component of the hemidesmosome, an epithelial-cell–connective-tissue attachment device, is thought to be involved in cytoskeleton–cell-surface anchorage. This 230 kDa polypeptide is recognized by bullous pemphigoid auto-antibodies and for this reason is generally termed the bullous pemphigoid antigen (BPA). We have identified two distinct mRNA products of the single BPA gene by RACE (rapid amplification of cDNA ends)/PCR techniques. The first of these mRNAs encodes the 230 kDa protein component of the hemidesmosome. A second mRNA lacks over 1800 bases that encode the C-terminus of the 230 kDa protein. We have raised antibodies against a peptide specific to the predicted protein product of this second mRNA. To our surprise this antibody recognizes a protein that migrates at 280 kDa on SDS/PAGE of extracts of

a variety of human epidermal cell lines that also express the 230 kDa BPA. Moreover, we have confirmed the co-expression of the 230 and 280 kDa polypeptides in these cells by immunoblotting analyses using a monoclonal antibody preparation directed against a polypeptide encoded by sequence common to both mRNAs transcribed from the BPA gene. Intriguingly, in one non-epidermal tumour line (a pancreatic cell line termed FG), the 280 kDa polypeptide appears to be the only product of the BPA gene. Furthermore, in FG cells the 280 kDa protein is found in association with the intermediate filament cytoskeleton. We discuss our results in relation to control of BPA gene expression and with regard to potential functions of the domains of the protein products of the BPA gene.

INTRODUCTION

The structural and associated polypeptides of the cytoskeleton often exist in multiple isoforms. These isoforms can result from post-translational modifications such as phosphorylation, acetylation and isoprenylation. Isoform diversity may also arise by RNA processing such as alternative splicing. There are two examples of the latter phenomenon that are particularly relevant to this study. Alternative splicing gives rise to different isoforms of desmoplakin, a component of the cell–cell attachment junction called the desmosome, and may result in production of two variants of the $\beta 4$ integrin subunit component of the hemidesmosome, an adherens junction involved in the attachment of epithelial cells to the underlying connective tissue (Staehelein, 1974; Tamura et al., 1990; Quaranta and Jones, 1991; Virata et al., 1992).

We present evidence that at least two mRNAs can be produced from the single-copy gene encoding a 230 kDa hemidesmosomal plaque protein (Sawamura et al., 1990; Amagai et al., 1991). The 230 kDa protein has been identified and characterized at the cell and molecular levels using autoantibodies found in the serum of patients afflicted with a blistering skin disease called bullous pemphigoid (BP) (Stanley et al., 1981, 1988; Klatte et al., 1989; Sawamura et al., 1991; Tanaka et al., 1991). Immunoelectron microscopy has revealed that the 230 kDa polypeptide or bullous pemphigoid antigen (BPA) is located within the cytoplasmic plaque of the hemidesmosome, in a region with which keratin-containing intermediate filament bundles of the epithelial cell interact (Klatte et al., 1989; Jones and Green, 1991). The latter finding has led, in part, to speculation that the 230 kDa protein

anchors keratin bundles to the hemidesmosome (Jones and Green, 1991).

Here we show that at least two mRNAs are transcribed from the BPA gene. We presume that production of the mRNA encoding the second BPA gene product impacts on the ability of a cell to produce the 230 kDa protein component of the hemidesmosomal plaque. We also discuss our results with regard to the function of the C-terminus of the 230 kDa polypeptide in the hemidesmosome.

MATERIALS AND METHODS

Cell lines and tissue specimens

Normal human keratinocytes, purchased from Clonetics Corp. (San Diego, CA, U.S.A.), SCC12 and SCC13 cells (derived from squamous cell carcinomas and provided by Dr. Amy Paller, Northwestern University Medical School), were maintained in keratinocyte growth medium (KGM; Clonetics Corp.). FG cells, a human pancreatic carcinoma cell line (provided by Dr. Vito Quaranta, Scripps Research Institute, La Jolla, CA, U.S.A.), were grown as detailed by others (Tamura et al., 1990). ENSON, a human fibroblast line provided by Dr. Robert Goldman (Northwestern University Medical School), were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (Gibco/BRL, Grand Island, NY, U.S.A.). Cells to be processed for immunofluorescence microscopy were plated on to glass coverslips coated with rat tail collagen (Collaborative Research, Bedford, MA, U.S.A.).

Human skin samples were provided by Dr. Frank Carone (Northwestern University Medical School). Skin material was

Abbreviations used: BP, bullous pemphigoid; BPA, bullous pemphigoid antigen; RACE, rapid amplification of cDNA ends; MMLV, Moloney Murine Leukaemia Virus.

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briefly incubated at 56 °C in PBS (30 s) prior to mechanical removal of the epidermal layers from the underlying connective tissue.

cDNA library screening

A λ gt11 human keratinocyte library was purchased from Clontech (La Jolla, CA, U.S.A.) and screened using a human BP serum sample as detailed previously (Hopkinson et al., 1992). A plasmid cDNA library produced from mRNA isolated from FG cells was provided by Dr. Vito Quaranta (Scripps Research Institute). This library was screened with a cDNA labelled by random priming using a kit supplied by Stratagene (La Jolla, CA, U.S.A.). Positive clones were taken through three rounds of colony purification, and plasmid DNA was isolated by standard techniques (Sambrook et al., 1989).

mRNA isolation

Poly(A)⁺ RNA was isolated from human epidermal/epithelial cells or the epidermal layers of human skin using the FastTrack or MicroFast Track mRNA Isolation Kit from Invitrogen (San Diego, CA, U.S.A.).

PCR amplification

cDNA was synthesized from various mRNA preparations by reverse transcription with Moloney Murine Leukaemia Virus reverse transcriptase (MMLV-RT) using an oligo(dT) primer and a First Strand Synthesis kit from Stratagene. cDNAs were amplified by PCR using the primers A (5'-d caagcaccgagttacagataac), B1 (5'-d ctgaacatgcatgatcacatc) and B2 (5'-d cttagacatgtagtagctgataaggtctc) (where 'd' indicates deoxy). PCR products were cloned into a *Sma*I-cut M13mp18 and sequenced with the Sequenase kit purchased from USB (Cleveland, OH, U.S.A.). 3' RACE (rapid amplification of cDNA ends) PCR was performed using mRNA isolated from various cell lines by reverse transcription with MMLV-RT (First Strand Synthesis kit; Stratagene) using RACE primer 1 (5'-d ctgactcgagtcgacatcgattttttttttttt). The cDNA was amplified by PCR using primer A and RACE primer 2 (5'-d ctgactcgagtcgacatcga).

Southern blot analysis of PCR products

PCR products obtained using the 3' RACE protocol were separated by electrophoresis through a 10% polyacrylamide gel and transferred to nitrocellulose filters by electroblotting. The filter was probed with primer P (5'-d gaatgtatctccagattaac) labelled with dCTP using terminal transferase (Boehringer Mannheim, Indianapolis, IN, U.S.A.). Following washings, the filter was exposed for autoradiography for 8 h and developed as above.

Isolation and PCR amplification of genomic DNA

Genomic DNA was isolated from FG cells by standard procedures (Sambrook et al., 1989). Genomic DNA was amplified by PCR using primers A and B1 (see above). The PCR product obtained was electrophoresed through a 10% polyacrylamide gel, and the fragment was excised from the gel, subcloned into the *Sma*I site of M13mp18 and sequenced using the Sequenase kit from USB.

Antibodies

The 5E human monoclonal antibody preparation was kindly provided by Dr. T. Hashimoto, Keio University School of

Medicine, Tokyo, Japan (Hashimoto et al., 1993). Serum from a BP patient containing autoantibodies against the 230 kDa BP antigen was provided by Dr. Ruth Freinkel (Northwestern University Medical School), and has been used in previous studies (Klatte et al., 1989; Hopkinson et al., 1992). A rabbit antiserum against keratin was obtained from Dr. Robert Goldman (Northwestern University Medical School).

A plasmid originally isolated from the FG cDNA library was partially digested with *Xba*I and the resultant 290 bp fragment was cloned into the *Nhe*I site of the vector pET11a for expression in *Escherichia coli* strain BL21(DE3) purchased from Novagen (Madison, WI, U.S.A.). For induction of the recombinant polypeptide, cells were grown overnight, then diluted 1:50 and allowed to reach an A_{600} of 1.0. At this time isopropyl β -thiogalactopyranoside (IPTG) was added to a final concentration of 1.0 mM and cells were harvested after a further 4 h of growth. Cells were pelleted and solubilized in a 8 M urea/1% SDS-containing buffer (Riddelle et al., 1991). This whole-cell extract was processed for SDS/PAGE (see below). The resulting gel was stained with Coomassie Brilliant Blue and the pET induced protein was excised, homogenized in PBS and used to immunize mice. Cells from the spleens of immunized mice were fused with the myeloma cell line Sp2, and monoclonal antibody-producing hybridomas were derived as detailed elsewhere (Harlow and Lane, 1988). The nature of the antibody produced by such lines was assessed by immunoblotting on whole-cell extracts of human epidermal-derived cells (see below). An IgG-producing hybridoma cell line selected by this criterion was cloned twice by limited cell dilution.

A peptide comprising the sequence CEVYSVF was prepared commercially (Multiple Peptide Systems, San Diego, CA, U.S.A.). This peptide was coupled to keyhole limpet haemocyanin using the Inject Immunogen EDC Conjugation Kit purchased from Pierce (Rockford, IL, U.S.A.). Following purification using a gel filtration column, the conjugated peptide was injected into mice. At 3-weekly intervals the mice were bled. The sera of mice were tested by dot blotting on immobilized peptide coupled to BSA prepared using the Inject Immunogen EDC Conjugation Kit.

SDS/PAGE, Western Immunoblotting and Immunofluorescence

SDS/PAGE, immunoblotting and immunofluorescence were carried out as detailed elsewhere (Laemmli, 1970; Towbin et al., 1979; Zackroff et al., 1984; Hopkinson et al., 1992). Extracts of cultured cells were prepared according to Riddelle et al. (1991).

RESULTS

Isolation of a cDNA encoding a portion of the 230 kDa hemidesmosomal plaque polypeptide

Using a BP autoserum, we initially screened a λ gt11 human keratinocyte expression library. Autoantibodies that were epitope-selected from the fusion proteins produced by two clones showed reactivity with a 230 kDa polypeptide present in cultured human keratinocytes (results not shown). One such clone, termed BP1, contained a 1401 bp insert. The derived amino acid sequence encoded by this insert is identical to the region spanning residues 922–1389 in the BPA sequence that is detailed in Tanaka et al. (1991).

Evidence for multiple products of the BPA gene

A clone, termed BP2, was identified in a plasmid cDNA library prepared from poly(A)⁺ RNA isolated from the human epithelial

Figure 1 Structure of a portion of the BPA gene

The boundaries of an intron are marked by asterisks. The coding sequence of the BPFG mRNA continues through the splice site for a further 15 nucleotides (nucleotides 361–375). Primers A, B1, B2 and P used in PCR/RACE analyses are indicated by the dashed lines and arrowheads. The putative polyadenylation signal for the BPFG mRNA is underlined. The end of the BPFG mRNA is marked by +.

cell line FG. This library was probed with the BP1 cDNA probe. BP2 contains an 806 bp insert which was subcloned into M13mp18 and M13mp19 to allow sequencing in both directions (Figure 1). The derived amino acid sequence encoded by the first 360 nucleotides of the insert show complete identity with residues 1342–1462 in the sequence of BP230 detailed in Tanaka et al. (1991). In contrast, the sequence of the BP2 insert between nucleotides 361 and 786 is distinct from the sequence detailed in Tanaka et al. (1991). Moreover, the BP2 insert contains a single open reading frame comprising nucleotides 1–375, followed by a TGA stop codon and 411 residues of untranslated region. A putative but unusual polyadenylation signal (AATATA) begins at nucleotide 766 (Birnstiel et al., 1985). A poly(A)⁺ tail of 20 nucleotides is located 11 residues downstream from this signal. For the purposes of clarity, we will call the putative mRNA containing the BP2 insert BPFG, while the 'conventional' mRNA will be termed BP.

To determine whether both BP and BPFG mRNAs are present in FG cells, amplification of DNA by PCR was performed with cDNA synthesized from mRNA isolated from these cells. In our

Figure 2 PCR amplification of FG genomic DNA and cDNA

(a) PCR products amplified from FG cDNA using primers A/B1 and primers A/B2 are shown in lanes 1 and 2 respectively. The PCR products were electrophoresed through a 10% polyacrylamide gel and stained with ethidium bromide. Molecular size standards are visible in lane S and are, from bottom to top, 50, 100, 200, 300, 400, 500, 700 and 1000 bp. Note the bands at 350 and 200 bp in lanes 1 and 2 respectively. There is also a minor species of approx. 1000 bp in lane 1. (b) The products of amplification of FG genomic DNA using primers A/B1 or primers A/B2 are shown in lanes 1 and 2. PCR products were electrophoresed through a 10% polyacrylamide gel and stained with ethidium bromide. Molecular size standards of 200, 300, 400, 500, 700 and 1000 bp (from bottom to top) are visible in lane S.

studies, we used three oligonucleotide primers whose nucleotide sequences are detailed in the Materials and methods section and are indicated in Figure 1. The sequence of one primer (A) is common to both the BP and BPFG mRNAs. Primer B1 is the complement of nucleotides 1102–1123, which are unique to the coding region of the BP mRNA. Primer B2 is sequence unique to the BPFG mRNA. As shown in Figure 2(a), fragments of 350 and 200 nucleotides are the PCR products generated by these two sets of primers. The size of these PCR products is that predicted if both BP and BPFG mRNAs are present in the cells. It should be noted that there is an additional minor PCR product of approx. 1000 bp generated by primers A and B1 (Figure 2a).

Characterization of a portion of the genomic structure of the BPA gene

Two different groups have shown that the 230 kDa polypeptide is encoded by one gene (Sawamura et al., 1990; Amagai et al., 1991). Thus it could be presumed that both the BP and BPFG mRNAs are encoded by the single BPA gene. To determine if this is the case, the genomic structure of a portion of the BPA gene was analysed. Using standard procedures, genomic DNA was isolated from the FG cells. Primer sets A/B1 and A/B2 were used to amplify fragments of this genomic DNA. Primer pair A/B1 produces a PCR fragment of approx. 1000 nucleotides, while the primers A/B2 yield a product of 200 nucleotides (Figure 2b).

In order to characterize the 1000 bp PCR product, the fragment was sequenced. The sequence begins at nucleotide 197 and ends at nucleotide 1123 in Figure 1. Sequence analyses reveal intron–exon borders with consensus donor and acceptor sites at nucleotides 361 and 926 respectively (Figure 1). However, the reading frame of the BPA gene continues for an additional 15 nucleotides following nucleotide 360 as shown in Figure 1. Indeed, the BPFG mRNA is the product of read-through across the splice site and into the intron. It should be noted that the sequence from nucleotide 928 to the end of the genomic PCR fragment (nucleotide 1123) matches exactly the sequence of the 230 kDa cDNA detailed by Stanley et al. (1988).

Figure 3 PCR products amplified from cDNA prepared from SCC12 cells (lanes 1 and 2), ENSON cells (lanes 3 and 4) and human epidermis (lanes 5 and 6) using primers A/B1 (lanes 1, 3 and 5) or primers A/B2 (lanes 2, 4 and 6)

Standards of 200, 300 and 1000 bp are indicated from bottom to top by circles next to lane 1. The PCR products were electrophoresed through a 10% polyacrylamide gel and stained with ethidium bromide. Note that there are no obvious PCR products in lanes 3 and 4. In lanes 1 and 5 there are products migrating at approx. 350 and 1000 bp. In both lanes 2 and 6 there is a PCR product of 200 bp.

Evidence for the presence of BP and BPFG mRNAs in other cell lines and tissues

We next asked the question whether BPFG mRNA is expressed in cells other than FG. For these studies, mRNAs from several human epidermal-derived cells (only the results using SCC12 cell mRNA are shown) and human skin were amplified by PCR using the primer sets A/B1 and A/B2 (Figure 3). In all cases, two PCR products of 350 and 200 nucleotides were observed. In contrast, no PCR products were generated using the above primers with mRNA isolated from the human fibroblast cell line ENSON (Figure 3).

Primers A/B1 generated a product of approx. 1000 nucleotides in addition to the 350-nucleotide product when using mRNA from both SCC12 cells and human skin (Figure 3). This is the same size as the product generated using FG cell genomic DNA as the template (Figure 2b). Thus this raises the possibility that our mRNA is contaminated with genomic DNA. To determine if this is the case, the isolated mRNA was treated with either RNAase-free DNAase or RNAase. Following phenol/chloroform extraction, cDNA was synthesized and each sample was processed for PCR using primer set A/B1. No PCR products were produced from the RNAase-treated sample, but both the 350- and 1000- nucleotide products were obtained in the DNAase-treated specimen (results not shown).

The above data suggest that the presence of the larger PCR product in Figures 2 and 3 is presumably not the result of amplification of genomic DNA contamination, but rather that there is a substantial amount of unprocessed mRNA in the cells. Because the 3' end of the BPFG mRNA is the result of read-through of a splice site into an intron of the BPA gene, it is possible that the 200-nucleotide PCR products seen in Figures 2 and 3 may be the result of priming of the unprocessed mRNA. Thus, in order to determine whether the BPFG mRNA actually exists in a variety of cell types, it was necessary to develop a method of identifying fully processed BPFG mRNA. We therefore utilized the RACE protocol to identify those products that are polyadenylated (Frohman, 1990). This technique would allow us to distinguish between the unprocessed mRNA and the processed, polyadenylated BPFG mRNA. The mRNAs of several cell lines (NHEK, SCC12 and SCC13) and human skin were

Figure 4 Amplification of cDNAs from NHEK, SCC12 and SCC13 cells and human skin using 3' RACE

PCR products obtained were electrophoresed through a 10% polyacrylamide gel and electroblotted on to nitrocellulose. The membrane was probed with end-labelled primer P and exposed overnight for autoradiography. A single reactive fragment migrating at approx. 600 bp is indicated by the arrow. Lanes: A, NHEK cells; B, SCC12 cells; C, SCC13 cells; D, human skin.

Figure 5 Western blot analysis of extracts of FG cells (lanes A, C, E and G) and SCC12 cells (lanes B, D, F and H)

Lanes A and B are the Amido Black stains of proteins transferred from a gel to nitrocellulose. Molecular mass markers of 200, 116, 96 and 63 kDa are indicated by the circles next to lane A. Lanes C and D were blotted with the m6 anti-peptide serum; lanes E and F were blotted with the 5E human monoclonal antibody; lanes G and H were blotted with 10C5 monoclonal antibody. Note that the m6 antibodies recognize 280 kDa proteins in both the FG and SCC12 cell extracts (lanes C and D; this polypeptide is indicated by the upper arrowhead next to lane H). A polypeptide of the same molecular mass is recognized by the 10C5 antibodies (lanes G and H). In addition, the 10C5 antibodies recognize a 230 kDa polypeptide in the SCC12 cell extract only (lower arrowhead, lane H). A similar 230 kDa protein is recognized in SCC12 cells by the 5E antibodies (this blot was overdeveloped to ensure that there was no reactivity of the 5E antibodies with the 280 kDa polypeptide) (lane F).

reverse-transcribed using a primer consisting of an oligo(dT) (17 residues) linked to a 20-nucleotide RACE fragment (RACE primer 1). Using primer A and RACE primer 2, the resulting cDNAs were amplified and the products processed for Southern blotting. A radiolabelled oligonucleotide (P; see the Materials and methods section and Figure 1) that encodes a sequence predicted to be contained within the RACE product reacts with an approx. 600-nucleotide fragment in the RACE products of NHEK, SCC12 and SCC13 cells as well as human skin (Figure 4). The RACE product of SCC12 cells was excised from a gel, subcloned into M13 and sequenced. The 619-nucleotide sequence

Figure 6 Immunofluorescence staining of SCC12 and FG cells

SCC12 cells (**a**, **b**) were prepared for single-label indirect immunofluorescence using 10C5 monoclonal antibodies. FG cells were prepared for double-label immunofluorescence using 10C5 antibodies (**c**) and an anti-keratin antiserum (**d**). Panels (**b**) and (**e**) show the phase images of the processed cells. In the group of SCC12 cells in (**a**), the 10C5 antibodies generate staining towards the periphery of each cell at the cell–substrate interface. In contrast, the same antibodies generate filamentous cytoplasmic staining in the FG cell shown in (**c**). These ‘filaments’ are recognized by anti-keratin antibodies (**d**). Bar in (**b**), 20 μm ; bar in (**e**), 10 μm .

obtained was identical with the sequence comprising nucleotides 197–786 in Figure 1, followed by a 20-residue poly(A)⁺ tail.

The protein product of BPFG mRNA

The putative protein product of the BPFG mRNA possesses a distinct C-terminus compared with that of the ‘conventional’ 230 kDa protein. An antibody (m6) was generated against a synthetic peptide composed of seven amino acids (CEVYSVF) located at the C-terminus of the BPFG mRNA product. The VYSVF sequence in this peptide is unique to the protein product of the BPFG mRNA and does not occur in the protein product of the BP mRNA. The additional two residues were included for ease of conjugation to the carrier protein keyhole limpet haemocyanin. The conjugate was injected into mice. As a marker for BP230 in our blots, we used a human monoclonal antibody, 5E, whose epitope has been mapped to a region within the C-terminus of BP230 (Hashimoto et al., 1993). This region is not present in the protein product of the BPFG mRNA. In addition, we generated a mouse monoclonal antibody (an IgG termed 10C5) against a pET fusion protein whose sequence is encoded by a region (nucleotides 1–289 in Figure 1) common to both the protein products of the BP and BPFG mRNAs.

Whole-cell extracts of FG and SCC12 cells were immuno-

blotted using 5E, m6 (the anti-peptide mouse serum) and 10C5. Antibodies in the m6 serum recognized polypeptides of about 280 kDa in both SCC12 and FG cells, although it should be pointed out that there was weak reactivity with several lower-molecular-mass species in these cell preparations (Figure 5). The reactivity of the m6 antibodies with all of these polypeptides was lost if the anti-peptide serum was absorbed against peptide conjugated to BSA. The 10C5 monoclonal antibodies recognized a protein of 280 kDa present in both the FG and SCC12 cells (Figure 5). These polypeptides co-migrated with species that are recognized by the m6 antibody preparation (Figure 5). In addition, 10C5 antibodies recognized a 230 kDa polypeptide that appears only in SCC12 cells (Figure 5). A protein of identical molecular mass in SCC12 cells was also recognized by the 5E antibodies. It should be noted that the reactivity of 5E is limited to the 230 kDa protein in the SCC12 cell preparation. The 5E antibodies failed to react with any protein in the FG cells. Identical immunoblotting results to those seen above were obtained if extracts of NHEK or SCC13 cells were used in place of the SCC12 cells (results not shown).

SCC12 and FG cells were analysed by immunofluorescence using those antibodies that were used in our immunoblotting studies. No obvious staining of either cell type was observed using the m6 serum sample (results not shown). The human

monoclonal antibody preparation 5E failed to stain FG cells, while the same antibodies generated intense substratum-associated staining of SCC12 cells, in a manner consistent with the localization of BP230 reported by others in the same cell (Hopkinson et al., 1992; results not shown). The 10C5 antibodies showed identical staining to the 5E antibodies in SCC12 cells (Figure 6a). In contrast, in FG cells the 10C5 antibodies stained a filamentous array that is not restricted to the substratum-attached surface of the cells (Figure 6c). Moreover, the filaments in FG cells that were stained by 10C5 antibodies were also recognized by anti-keratin antibodies (Figure 6d).

DISCUSSION

In this study we have identified two distinct mRNAs transcribed from the single gene encoding a 230 kDa polypeptide component of the hemidesmosome. One mRNA (BP) has previously been identified by Stanley et al. (1988). A second mRNA (BPFG) was first detected in FG cells. However, its existence in normal human keratinocytes (in tissues as well as *in vitro*) and in their transformed counterparts has been confirmed by PCR/RACE analyses.

There are several possible mechanisms by which the BPFG mRNA is produced. For example, BPFG mRNA may be produced by premature termination of transcription. In eukaryotes this mechanism is involved in the regulation of immunoglobulin, *c-fos*, *c-myc* and adenosine deaminase gene expression (Weiss et al., 1989, 1991; Maa et al., 1990; Mechti et al., 1991; London et al., 1991). Alternatively, BPFG mRNA may simply be produced by differential RNA processing of the primary transcript.

One unexpected aspect of our studies was the detection of considerable quantities of unprocessed mRNA of the BP gene in all cells and tissues used for PCR analysis. In fact, in human skin, our PCR analyses indicate that there are relatively small levels of processed mRNA compared with unprocessed mRNA. This may reflect another important level of regulation of expression of the BP gene.

We have prepared antibodies against a peptide that is encoded by the BPFG mRNA and antibodies that recognize a domain that we predict is contained in both the protein products of BP and BPFG mRNAs. We were also fortunate to have a human monoclonal antibody (5E) that is specific to the product of the BP mRNA (Hashimoto et al., 1993). These have allowed us to distinguish between the protein products of the BP and BPFG mRNAs. Our results in this regard are quite surprising. Translation of the BPFG mRNA is predicted to produce a polypeptide which lacks 625 amino acid residues of the C-terminus of the 'conventional' 230 kDa polypeptide. Yet, using our peptide serum m6, we show that the BPFG mRNA apparently encodes a 280 kDa polypeptide, i.e. 50 kDa larger in molecular mass than the conventional 230 kDa protein. There are several possible explanations for this apparent contradiction. First, both the BP and BPFG protein products may migrate anomalously on SDS/polyacrylamide gels. Certainly this is consistent with molecular analyses revealing that the conventional 230 kDa protein of the hemidesmosome is higher in molecular mass (at least 300 kDa) than is indicated by SDS/PAGE (Sawamura et al., 1991). A more likely possibility is that there are other distinctions between the coding regions of the BP and BPFG mRNAs 5' to the region that we have so far been able to analyse. These obviously would encode additional unique domains specific to the protein product of BPFG mRNA. The nature of these domains may be revealed once more detailed analyses of the genomic structure of the BPA gene have been presented.

In several recent studies, workers have made predictions concerning the secondary structure of the deduced amino acid sequence of the 230 kDa protein product of the BP mRNA. Green et al. (1990) and Tanaka et al. (1991) have reported that the C-terminal one-third of the 230 kDa polypeptide contains two domains, termed B and C, which contain 174 and 176 amino acids respectively. These domains show sequence similarity to each other. More importantly, they also show identity to repeats present in the C-termini of both desmoplakin I, a component of the desmosome, and plectin, an intermediate-filament-associated protein (Green et al., 1990; Wiche et al., 1991). Indeed, these proteins have been proposed to belong to a common family of intermediate filament-associated proteins (Wiche et al., 1991; Green et al., 1992). It has been hypothesized that all three proteins interact with intermediate filaments via these domains, since the sequence of the latter shows the same periodicity in acid and base residues as do intermediate filament subunits, although direct biochemical evidence to this effect is lacking (Green et al., 1990).

The 280 kDa polypeptide product of the BPFG mRNA does not possess the B and C domains of the C-terminus of the conventional 230 kDa protein that we have mentioned above (Green et al., 1990; Tanaka et al., 1991). Thus, based on the predictions of Green et al. (1992), it might be supposed that the 280 kDa polypeptide would not interact with intermediate filaments. However, this does not appear to be the case. Despite the lack of reactivity of the m6 serum antibodies at the fluorescence level, 10C5 antibodies show dramatic staining of both the FG and SCC12 cells. It should be remembered that the 10C5 antibody preparation was generated against a domain that is common to both the protein products of the BP and BPFG mRNAs. Furthermore, the 10C5 monoclonal antibodies recognize only the 280 kDa protein in whole-cell extracts of FG cells, as assessed by immunoblotting. Therefore the staining pattern that the 10C5 antibodies generate in FG cells presumably represents the distribution of the protein product of BPFG mRNA only, since we can find no immunochemical evidence for the existence of the 230 kDa protein in the FG cells using either the 10C5 or 5E antibody preparations. Thus our fluorescence analyses imply that the BPFG protein product, at least in FG cells, is associated with the keratin-containing intermediate filament system even though it lacks the B and C domains of the 230 kDa/desmoplakin/plectin family of polypeptides.

There remains one puzzling feature of our fluorescence studies. 10C5 antibodies generate staining that is restricted to the substratum-attached surfaces of SCC12 cells. This localization pattern is identical to that seen using 5E antibodies that are specific for the 230 kDa protein product of the BPA gene. However, like FG cells, SCC12 cells possess the protein product of the BPFG mRNA. Based on the patterns of fluorescence generated by 10C5 antibodies in FG cells, one could assume that the 280 kDa protein co-distributes with the keratin networks of the SCC12 cells. This is not the case.

In summary, we have shown that the BPA gene encodes a second protein of molecular mass 280 kDa by SDS/PAGE. Both the 230 and 280 kDa polypeptides are expressed in cultured transformed as well as normal human epidermal cells. We also present data showing that both BP and BPFG mRNAs are expressed in normal human skin. We speculate that the large amounts of unprocessed mRNA derived from the BP gene, particularly evident in tissues, are somehow involved in the regulation of expression of the gene. Moreover, it appears likely that the production of BPFG mRNA also plays a role in the regulation of expression of BP mRNA. Differential expression of the BP mRNA and therefore of its 230 kDa protein product

presumably impacts on the ability of a cell to assemble a hemidesmosome. It should be borne in mind that the cell type in which we detected only the 280 kDa protein product of the BP gene is a cell line derived from a pancreatic carcinoma. Whether up-regulation of the BPFGE mRNA is triggered during carcinogenesis, and whether this correlates with the decreases in the numbers of hemidesmosomes that have been observed in transformed cells, should be an interesting avenue of study (McNutt, 1976; Jones et al., 1989).

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