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Identification of BCAP, a new protein associated with basal bodies and centrioles

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1. ABSTRACT

Cilia exert critical functions in numerous organisms, including that of cell motility, fluid transport and protozoan locomotion. Defects in this organelle can lead to lethal pathologies in humans, including primary ciliary dyskinesia. An understanding of the cilia formation process would lead to better characterization of defects involved in such pathologies. In the present study, we identified a gene encoding a novel human protein, BCAP for Basal body Centriole-Associated Protein, which shares homologies with a previously described protein, Outer Dense Fiber 2 (ODF2). ODF2, a major component of the sperm tail cytoskeleton, is required for the formation of mother centriole distal/subdistal appendages and the generation of primary cilia. Here, we show that the *bcap* gene contains 18 alternatively spliced exons and encodes five different isoforms, three long and two short ones. BCAP is preferentially expressed in cilia/flagella containing tissues. Moreover, its expression is correlated with cilia formation during mucociliary differentiation of human nasal epithelial cells. Using immunofluorescence analyses, BCAP was localized within basal bodies of ciliated cells and within centrioles of proliferating cells. In light of the several spliced isoforms of BCAP and the particular localization of the protein, BCAP isoforms could play distinct roles in cilia and in centrosomes.

2. INTRODUCTION

Cilia and flagella play essential motile and sensory functions in tissues, including sperm cell motility, transport of mucus along the respiratory tract and stirring of cerebrospinal fluid. Defects in cilia formation or function, i.e. ciliary beating, like the immotile cilia syndrome and Primary Cilia Dyskinesis (PCD), are important causes of pulmonary pathologies (1) and may ultimately result in chronic pulmonary disease characterized by bronchiectasis. In addition, the absence of functional cilia may lead to male sterility. Ciliogenesis is also important in left-right axis patterning during embryogenesis, heart development, maintenance of the renal epithelium and electrolyte balance in the cerebrospinal fluid. Given the wide range of cilia and flagella functions, it is essential to understand molecular processes involved in ciliogenesis. Cilia formation can proceed through two different pathways. In epithelial cells, ciliogenesis is a terminal cell differentiation process mainly observed in respiratory and reproductive tracts. Procentrioles are assembled around electron-dense cytoplasmic granules near the Golgi area. Centrioles elongate until they are full-length, then migrate via an actin-dependent mechanism and anchor to the apical membrane where they trigger cilia formation. Thus, centriole/basal bodies serve as templates for the assembly of microtubule doublets of the axoneme, characterized by nine microtubule pairs surrounding a central pair (referred

to 9+2 structure). A large variety of proteins are localized within the periphery of the centrioles/basal bodies as part of the pericentriolar material. In contrast, in ciliated protozoans, basal bodies are not assembled *de novo* but duplicate: procentrioles elongate perpendicularly to their parents and then anchor to the cortex prior to cilia assembly, resembling a semi-conservative process described for centrosome duplication in proliferating cells. Indeed, centrioles are also present in the centrosome of animal cells and certain structural and regulatory proteins, including tubulins and centrins, are common to both organelles. Recent genomic analyses led to identification of genes specifically expressed in ciliated organisms (2, 3) and proteomic studies enabled identification of proteins specifically localized within centrioles (4, 5) and within cilia (6). Nevertheless, most of these new centriolar and cilia components remain uncharacterized. Among proteins described as being centrosomal components, Outer Dense Fiber 2 (ODF2; also known as cenexin), initially identified as a main component of the sperm tail cytoskeleton, is a general scaffold protein specifically localized at the distal/subdistal appendages of mother centrioles (7), and it is probably a new microtubule-associated protein (8). Moreover, ODF2 silencing in mouse F9 cells did not lead to a modification of cell cycle progression, nor of cell appearance; but it did reveal the disappearance of distal/subdistal appendages. Thus, ODF2(-/-) cells cannot trigger primary cilia formation. These results suggested a central role for ODF2 in anchorage of the centriole/basal body. Without appendages, the basal body does not seem to dock with the plasma membrane (9).

Using differential display performed on *in vitro* differentiating Human Nasal Epithelial (HNE) cells versus proliferative cells, we identified a set of known and unknown genes overexpressed during differentiation. Here, we describe a new alternatively spliced gene *bcap* encoding for Basal body Centriole-Associated Protein, which shares 20% similarity with ODF2 and which gives rise to five different mRNA. We show that BCAP isoforms are overexpressed during MucoCiliary Differentiation (MCD). Using specific polyclonal anti-BCAP antibodies, we demonstrate that part of BCAP is enriched both within centrosomes of proliferating cells and within centriole/basal bodies of HNE cells.

3. MATERIALS AND METHODS

3.1. Air-liquid interface (ALI) culture and suspension culture

Human nasal turbinates were obtained from patients who underwent polypectomy or turbinectomy (Hôpital Lariboisière and Hôpital La Salpétrière, Paris, France). Cell culture was achieved as described in Laoukili et al. for suspension cultures (10) and Million et al. for ALI cultures (11). Briefly for ALI cultures, after dissociation by an overnight pronase treatment, cells were preplatted on plastic flasks to eliminate fibroblasts. After filtration on a 30µm diameter filter, cells were plated at a density of 3.10^4 cells per cm^2 onto type I collagen-coated semipermeable membranes and cultivated until confluence in a 1/1 BEGM/DMEM/F12 (Clonetics [Biowhittaker, Emerainville, France], Gibco) mixture added at both apical and basolateral sides. At confluence (day 0), the ALI was created (medium present only at the basolateral side) and retinoic acid (RA) (Sigma) added to the culture medium induced mucociliary differentiation (MCD) whereas in the absence of (RA) epidermoid differentiation was observed.

3.2. Antibodies

These included: anti-ODF2, a mAb raised against the ODF2 protein (a gift from S. Tsukita, Kyoto University, Japan); CTR453, an anti-centrosome mAb (M. Bornens, Institut Curie, Paris, France); GT335, a mAb raised against a chemically glutamylated C-terminal peptide of alpha-tubulin and recognizing polyglutamylated tubulins present in centrioles/basal bodies and cilia formed during MCD (P. Denoulet, Université Paris 6, Paris, France); FITC-conjugated anti-rabbit (Dako, Glostrup, Denmark); TRITC-conjugated anti-mouse (Dako, Glostrup, Denmark); and peroxidase-conjugated anti-rabbit IgG (Dako, Glostrup, Denmark) and peroxidase-conjugated anti-mouse IgG (Dako, Glostrup, Denmark). To obtain antibodies directed against BCAP, a KLH-coupled synthetic oligopeptide (sequence: QEHTIRELQGQVDGN) was used. Rabbit immunization was performed according to Agrobio (La Ferté Saint Aubin, France) protocol.

3.3. cDNA differential display

Total RNA was extracted from cells by the acid phenol/guanidine thiocyanate method of Chomczynski and Sacchi (12). Poly(A+) RNA were isolated using an oligo-dT kit (Qiagen, Courtaboeuf, France) from epithelial cells at day 0 and at day 6 during MCD. Differential hybridization analyses were achieved with Clontech Atlas™ cDNA arrays (Clontech Laboratories, Mountain View, CA) containing 588 selected cDNAs on a nylon membrane. 32P-labelled complex probes were obtained from subtractions or from total cDNAs and hybridized to the cDNA array (see Clontech website). Hybridization and washes were performed at high stringency conditions.

3.4. Northern blotting

Probes were synthesized from PCR products with the Redyprime II DNA labelling system (Amersham Biosciences, Uppsala, Sweden) according to the manufacturer's instructions. Human 12-Lane MTN blot III and Human MTN blot II (Clontech Laboratories, Mountain View, CA) containing normalized amount of poly A+ RNA were prehybridized and hybridized in Rapid-Hyb solution (Amersham Biosciences, Uppsala, Sweden) and washed according to the manufacturer's instructions. Membranes were exposed to a phosphor screen and signal detected with a Storm 860 machine (Molecular Dynamics), and quantified using Bio 1D software (Vilber Lourmat Biotechnology).

3.5. RT-PCR

Total cellular RNA was prepared according to a method previously described (13). Equal amounts (2 µg) of total cellular RNA were reverse-transcribed using OligodT Primer and RevertAidTM H Minus M-MulV reverse transcriptase (Fermentas, Hanover, USA). PCR reactions

Table 1. Known genes upregulated during MCD

Family	Gene name	Uniprot	Function
Housekeeping	HC5	P20618	Proteasome subunit
	SAM50	Q9Y512	Import of mitochondrial protein
	Elongation factor 1□	P26641	Delivery of amino-acyl tRNA to the ribosome
	GNPAT	O15228	Peroxisomal protein implicated in etherlipids synthesis
	SPC18	P67812	Microsomal signal peptidase 18kDa subunit
	UCP2	P55851	Uncoupling oxidative phosphorylation from ATP synthesis
	ATP synthase □ chain	P06576	ATP synthesis
Cell cycle	RCC1	P18754	Regulator of chromosome condensation
	Cullin1	Q13616	Negative regulator of cell cycle, component of the E3 ubiquitin ligase complex
	SIAH1	Q8IUQ4	E3 ubiquitin ligase activated in apoptotic cells
Transcription and mRNA maturation	**ELF3**	**Q6IAP8**	**Epithelial specific transcription factor**
	TMF1	P82094	Modulation of transcription
	CPSF5	O43809	Maturation of mRNA
	CLOCK	O15516	Transcription factor implicated in circadian cycle
	TXBP151	Q13311	Anti-apoptotic transcription factor
	LRRFIP1	O75799	Transcription repressor
Cytoskeleton	Matrin 3	P43243	Internal nuclear matrix protein
	Hp28	**O14645**	**Ciliary/flagellar motility**
	Plastin 1	**Q14651**	**Actin bundling protein in the absence of calcium, localized in microvilli**
	UNC84	**O94901**	**Nuclear envelope protein implicated in nuclear migration**
Immunity/ inflammation	**PLUNC**	**Q9NP55**	**Innate immune response in nose, mouth, lung and trachea**
	Lipocalin 2	**P80188**	**Control of inflammation process in oral tissues**
	GCP2	**P80162**	**Inflammation mediator**
	Lipocortin	**P04083**	**Control of inflammation mediators synthesis**
	B94	**Q03169**	**Inflammation and angiogenesis mediator**
	Sialomucin	Q9NR26	Negative regulator of CD34+ progenitor proliferation
	HLA-DRA1	P01903	HLA II histocompatibility antigen
Diverse	PLAC8	Q9NZF1	Gene downregulated in dendritic cells derived from CD34+ progenitors
	MAC-MARCKS	P49006	PKC substrate implicated in neural tube formation
	ZNF185	O15231	LIM-domain zinc finger protein expressed in prostate, testis, ovary, placenta and peripheral blood
	NIF3L1BP1	Q6I9Y2	Implicated in neuronal differentiation
	SELT	P62341	Selenocystein containing protein
	PIP	P12273	Expressed in breast tumor and in some exocrine organs
	Synaptotagmin-like protein 5	Q8TDW5	Rab27A effector implicated in vesicular trafficking

Genes identified as overexpressed during MCD of HNE by cDNA differential display can be listed in several families regarding their already described functions. For each gene, the uniprot accession number and a summary of the function are indicated. Genes considered as relevant regarding MCD are in bold letters (see discussion).

were performed on 2 µl of cDNA using DyNAzyme EXT™ (Finzymes, Espoo, Finland). Amplification conditions were optimized. PCR products were analyzed on 1.5% agarose electrophoresis gels. Band intensity was compared with respect to the GAPDH mRNA amplification and quantified using Bio 1D software (Vilber Lourmat Biotechnology). Sequences used for each primer are available on request.

3.6. Protein analyses

Dissociated cells were directly resuspended in SDS-PAGE sample buffer. Samples were boiled and centrifuged at 10000 g for 5 min. The protein concentration was determined by microBCA (Pierce Biotechnology, Rockford, IL). SDS-PAGE were performed according to the method of Laemmli (14) on 10% polyacrylamide gels using normalized amounts of proteins. Transfer and immunodetection were performed as described by Paoletti et al. (15). The membranes were incubated with primary antibody diluted in TBS 0.1% Tween 20 and 3% milk for 1 h at room temperature. Blots were washed and incubated with peroxidase-labelled secondary antibody for 45 min at room temperature. After intensive washing, the membrane was incubated in ECL reagent (Amersham Biosciences, Uppsala, Sweden) before exposure to X-ray light-sensitive film.

3.7. Immunofluorescence

Epithelial cells were washed in PBS and fixed in methanol at -20°C for 6 min or in 3% paraformaldehyde. Alternatively, cells were first treated in PHEM (PIPES 45mM pH6,9, Hepes 45 mM pH6,9, EGTA 10 mM, $MgCl_2$ mM, PMSF 1 mM) buffer containing 0.06% Triton X-100 (16) for 30 sec, rinsed in PHEM buffer and fixed in methanol at -20°C. In all cases, immunofluorescences were performed as described (15). Finally, cells were washed in PBS, dehydrated in ethanol and mounted in citifluor for microscopy analyses.

4. RESULTS

4.1. Identification of genes upregulated during ciliated cell differentiation

To identify new genes that are upregulated during ciliogenesis, differential display using RNA from differentiating and proliferating HNE cells in suspension culture was carried out. 80 clones were obtained and partially sequenced. Sequence comparison showed that 34 of these genes were already known, while the others corresponded to EST found in databases. The known genes could be classified into 6 categories (Table 1). Among these categories, three groups were particularly relevant in

terms of MCD: the fourth group contained proteins associated with cytoskeleton, which is largely reshaped during MCD, including hp28, an axonemal dynein light chain; the group of immunity/inflammation genes including genes already described as being specific to the innate immune response of the nose (PLUNC) (17) or inflammation of the oral tissues (lipocalin 2) (18); and the sixth group, containing proteins associated with or modulated upon differentiation of other tissues.

Thus, to characterize new genes potentially implicated in cilia function, blast comparisons were performed using unknown sequences identified from the cDNA differential display. We selected a new gene that we named *bcap*. The BCAP protein shared homology with *H. sapiens* ODF2 (20% similarity), a gene coding for a protein localized within centrioles in proliferative cells (7) and implicated in ciliogenesis (9). It also shared homology with *S. cerevisiae* NUF1/SPC110 (45% similarity), a protein associated with the inner plate of the spindle pole body, the budding yeast functional homologue of the centrosome (19) and with ATPases implicated in segregation and maintenance of chromosomes (20). Moreover, BCAP is conserved among evolution. Homologues have been found in a large variety of species that possess cilia or flagella (Figure 1C). Indeed, BCAP homologues have been found in mammalian and vertebrate species.

4.2. The BCAP sequence predicts more than one protein

The BCAP gene is localized on the 1p22.3-31-2 locus. To determine the BCAP mRNA sequence, RACE-PCR experiments were performed to obtain additional clones in both the 5' and 3' direction from the original BCAP clone. Two variants of BCAP with different initiatior codon were detected in HNE cells: a long one, notified as L-BCAP for Long-BCAP and a short one notified as S-BCAP for Short-BCAP. *bcap* gene sequence contained 18 exons. S-BCAP initiatior codon was localized in exon 7, and exons 10 and 14 were specific for S-BCAP. They were not present in all L-BCAP isoforms (Figure 1A, 1B). The stop codon for L- and S-BCAP mRNA was the same and localized at the end of exon 18. L-BCAP sequence was found in ncbi protein databank as a novel protein (Whitehead, accession number: BAA86543). To further investigate the different forms of these two variants, RT-PCR using different pairs of primers on total extracted RNA from HNE cells were achieved and enabled to detect 5 mRNA forms. Three long mRNA forms showing alternative splicing for exons 2 and 13 (exons 10 and 14 were never detected in L-BCAP). Two short mRNA forms were also detected showing alternative splicing of exons 13 and 14 (Figure 1B).

4.3. The pattern of BCAP expression is tissue-specific

To examine BCAP tissue distribution, Northern blot analyses were carried out on poly A+ RNA corresponding to different human adult tissues (Clontech) using a specific 32P-labelled BCAP cDNA probe. This probe was an amplification product of exon 11 to exon 16 recognizing all BCAP mRNA. BCAP was mainly expressed in trachea and testis, two tissues containing motile cilia or flagella (Figure 2), while in other tissues mRNA was not detected (bone marrow, bladder, leucocytes, etc.) or only weakly detected (tongue, stomach, brain, ovaries, etc.). In testis, one transcript of 2.3 kb was detected, whereas two mRNA, corresponding to 2.3 and 2.8 kb, were observed in trachea. The presence of two transcripts with different sizes was compatible with transcription from alternative promoters. In the present experiment, we were not able to discriminate between different long and short isoforms, but the 2.8 kb transcript could correspond to L-BCAP, whereas the 2.3 kb transcript may correspond to S-BCAP. Nevertheless, this result suggested that all BCAP isoforms were restricted to tissues containing axonemal structures as confirmed by the detection of BCAP in western blot experiments in HNE cells during MCD (see below) and in human spermatozoa (data not shown).

4.4. BCAP expression is correlated with mucociliary differentiation

HNE cells were able to differentiate *in vitro* into ciliated cells in the presence of retinoic acid (RA) (Million et al., 2001). To understand the expression pattern of each BCAP variant during MCD, total RNA were extracted at different times (days 0, 8, 15 and 30) during differentiation in the presence or in the absence of RA, and semi-quantitative RT-PCR experiments were undertaken. L-BCAP mRNAs were amplified using two sets of primers localized in exons 1 and 13 and in exons 7 (before transcription initiation of S-BCAP, which contains a smaller exon 7) and 15, respectively. Primers hybridizing in exons 1 and 13 enabled visualization of L-BCAP and L-BCAP(del2), while primers hybridizing in exons 7 and 15 enabled visualization of L-BCAP and L-BCAP(del2), which can not be distinguished from each other, and L-BCAP(del13). In both cases, the upper band was the major form (Figure 3A). S-BCAP mRNA were amplified using primers localized in exons 10 and 16 (exons 10 and 14 were specific for S-BCAP). As shown for L-BCAP variants, S-BCAP was more abundant than S-BCAP(del13,14).

Semi-quantitative RT-PCR experiments were also performed using primers localized within exons 11 and 16 to detect all BCAP variants (Figure 3B) to show the mRNA level of accumulative L-BCAPs and S-BCAPs during MCD. We observed 2 bands: the upper one (band 1) corresponded to isoform without splicing between exons 11 and 16 (S-BCAP), and the lower one (band 2) corresponded to isoforms containing splicing of exons 13 and/or 14 (L-BCAP, L-BCAP(del13), L-BCAP(del2) and S-BCAP(del13,14), all these isoforms could not be distinguished from each others due to very weak differences in their size and to the small size, 27 bp, of exon 14). The BCAP mRNA level gradually increased during MCD of HNE cells (from day 8 to day 30 in the presence of RA). Transcripts were also detected in proliferative cells (day 0) and during epidermoid differentiation (in the absence of RA), but the level of transcripts was lower. Moreover, ODF2 mRNA expression also increased during MCD *in vitro* and remained low during epidermoid differentiation (Figure 3B). Figure 3C shows the relative quantities of band 1 (S-BCAP) and band

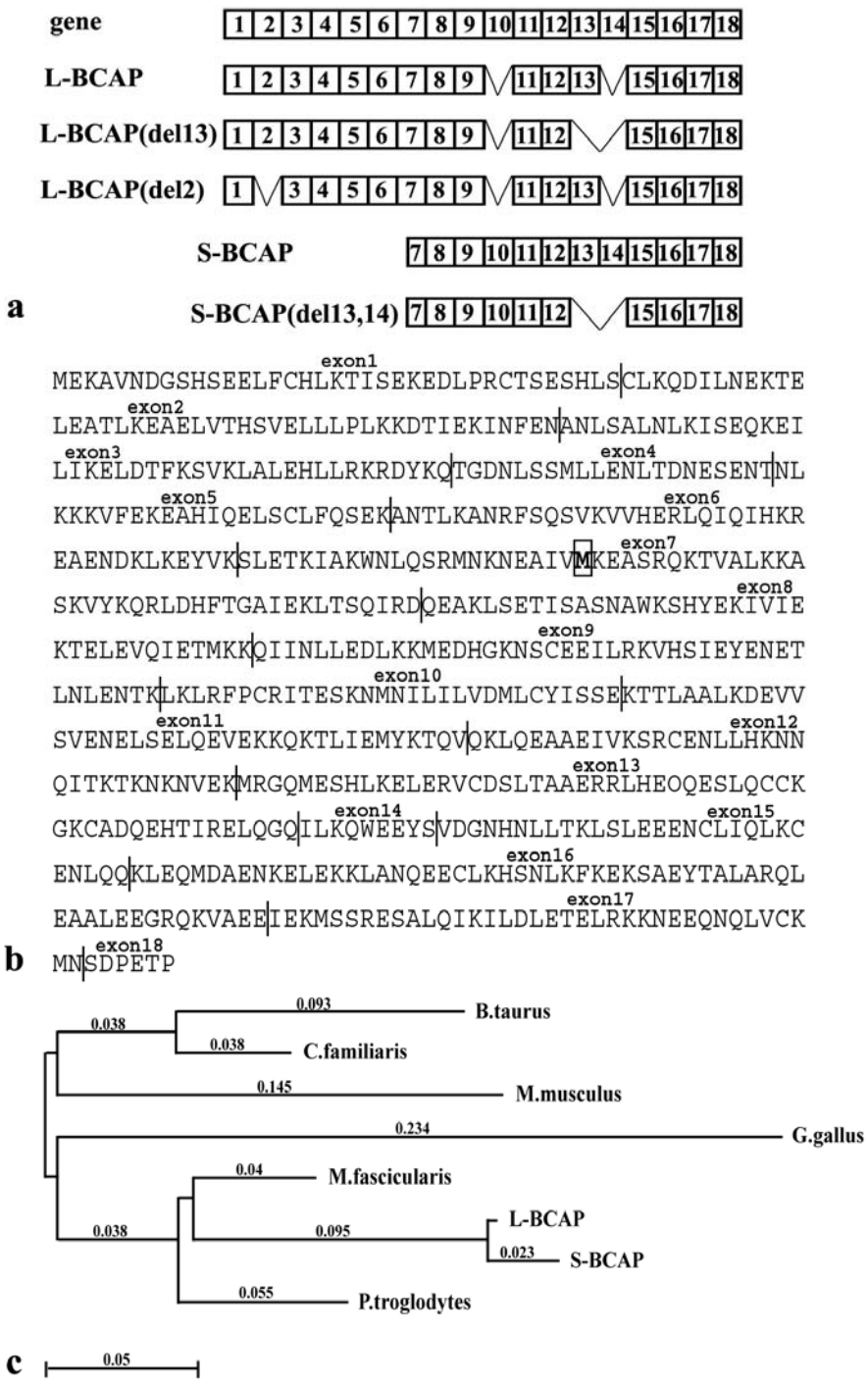

Figure 1. BCAP sequence and structure. (A) Schematic representation of BCAP mRNA. The BCAP gene potentially contains 18 exons. Combining the use of two alternative promoters and alternative splicing, it encodes 5 different mRNA: three long BCAP isoforms (L-BCAPs), which do not possess exons 10 and 14 (L-BCAP), and show alternatively splicing for exon 2 (L-BCAP(del2)) and 13 (L-BCAP(del13)); two short BCAP isoforms (S-BCAPs) that possess a shorter exon 7 and whose initiator codon is localized in exon 7. One S-BCAP variant is alternatively spliced for exons 13 and 14 (S-BCAP(del13,14)). (B) Translation of the 18 exons of the *bcap* gene. The initiator methionine for S-BCAPs is in bold and boxed, and specific exons for S-BCAP forms (exons 10 and 14) are in bold. (C) Midpoint rooted tree of BCAP homologues, showing phylogenetic distances, was obtained using ClustalW with the Blosum similarity matrix. Sequence accession numbers: *B. taurus* NP_001069461, *C. familiaris* XP_547300, *G. gallus* XP_422361, *M. fascicularis* BAE00647, *M. musculus* NP_079990, *P. troglodytes* XM_513532.

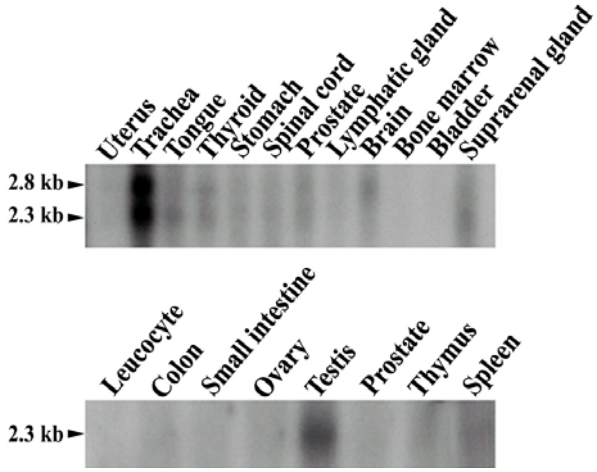

Figure 2. BCAP expression in human adult tissues. Different human tissues are Northern-blotted and incubated with a 32P-labeled BCAP cDNA probe. BCAP is widely expressed in trachea and testis. A major isoform corresponding to 2.3 kb transcripts is observed in both tissues. In trachea, a minor isoform corresponding to a 2.8 kb transcript is also observed, corresponding to an alternative spliced isoform or the use of an alternative promoter.

2 (L-BCAP, L-BCAP(del13), L-BCAP(del2) and S-BCAP(del13-14)) normalized versus the GAPDH amplification product. These diagrams clearly showed that accumulation of BCAP mRNA levels increased during MCD (white bars). The band 2 increase was not as high as that of band 1, but could be explained by expression regulation of the different isoforms during MCD.

To study BCAP expression during MCD, a polyclonal antipeptide antibody raised against and recognizing all BCAP isoforms was used in western blot experiments. Two polypeptides were detected in HNE cells (Figure 3D). 40 kDa S-BCAP expression gradually increased during MCD (+RA) and was reduced during epidermoid differentiation (-RA), whereas L-BCAP (65 kDa) only increased when cells were differentiated (day 30). In contrast, S-BCAPs were not detected in confluent cells, suggesting a small amount of these isoforms in proliferative cells. Ciliated cells, which were enriched at day 30, were detected by GT335 recognizing polyglutamylated tubulins. Clearly, S-BCAP expression was enhanced prior to detection of fully differentiated ciliated cells by GT335 mAb.

4.5. BCAP is enriched in centriole/basal bodies of HNE ciliated cells and within centrioles of proliferating cells

The cellular distribution of BCAP during MCD of HNE cells was assessed by indirect immunofluorescence analyses. Early during the MCD process, BCAP was enriched within the subapical region of epithelial cells prior to cilia formation (Figure 4A, day 15, arrow) reinforcing the idea that BCAP was expressed before basal body formation. Moreover, BCAP did not colocalize with GT335 in fully differentiated cilia (Figure 4B, arrowhead). No fluorescence signal was detected with the BCAP pre-immune serum (data not shown).

In situ immunostaining with the polyclonal antipeptide raised against BCAP showed a subapical localization in differentiated epithelial cells (data not shown). No staining was observed in basal cells or at the basolateral pole of epithelial cells. BCAP is thus a new protein strictly localized within the apical cytoplasmic domain of ciliated epithelial cells *in situ*, corroborating *in vitro* experiments.

To elucidate the low abundance of BCAP in non-cilated epithelial cells, RT-PCR and indirect immunofluorescence experiments were carried out using a human bronchial epithelial cell line, 16HBE. Two BCAP transcripts were detected in this cell line (data not shown) using primers localized in exon 11 and exon 16 suggesting the existence of S-BCAP and L-BCAP in this cell line. Double-labelling immunofluorescence experiments using anti-BCAP pAb and GT335 mAb revealed that BCAP was present at full-length and growing centrioles (Figure 5). This result was confirmed using CTR453, a monoclonal antibody, which specifically recognized human centrosomes. Finally, double-immunostaining with a monoclonal anti-ODF2 and anti-BCAP showed that the two proteins were not strictly colocalized within the centrosome.

5. DISCUSSION

Recent proteomic approaches to identify centriolar or ciliary components have listed different proteins involved in centrosome and cilia structures (4, 6). Moreover, two recent studies using a genomic approach highlighted cilia and basal body components conserved among different species (2, 3). Characterization of these components represents a double quest: understanding of the molecular mechanisms involved in the ciliogenesis process and identifying target genes mutated in a growing field of genetic human diseases. The critical role of ciliated cells in mucociliary clearance was clearly demonstrated by severe bronchitis and chronic sinusitis resulting from recurrent

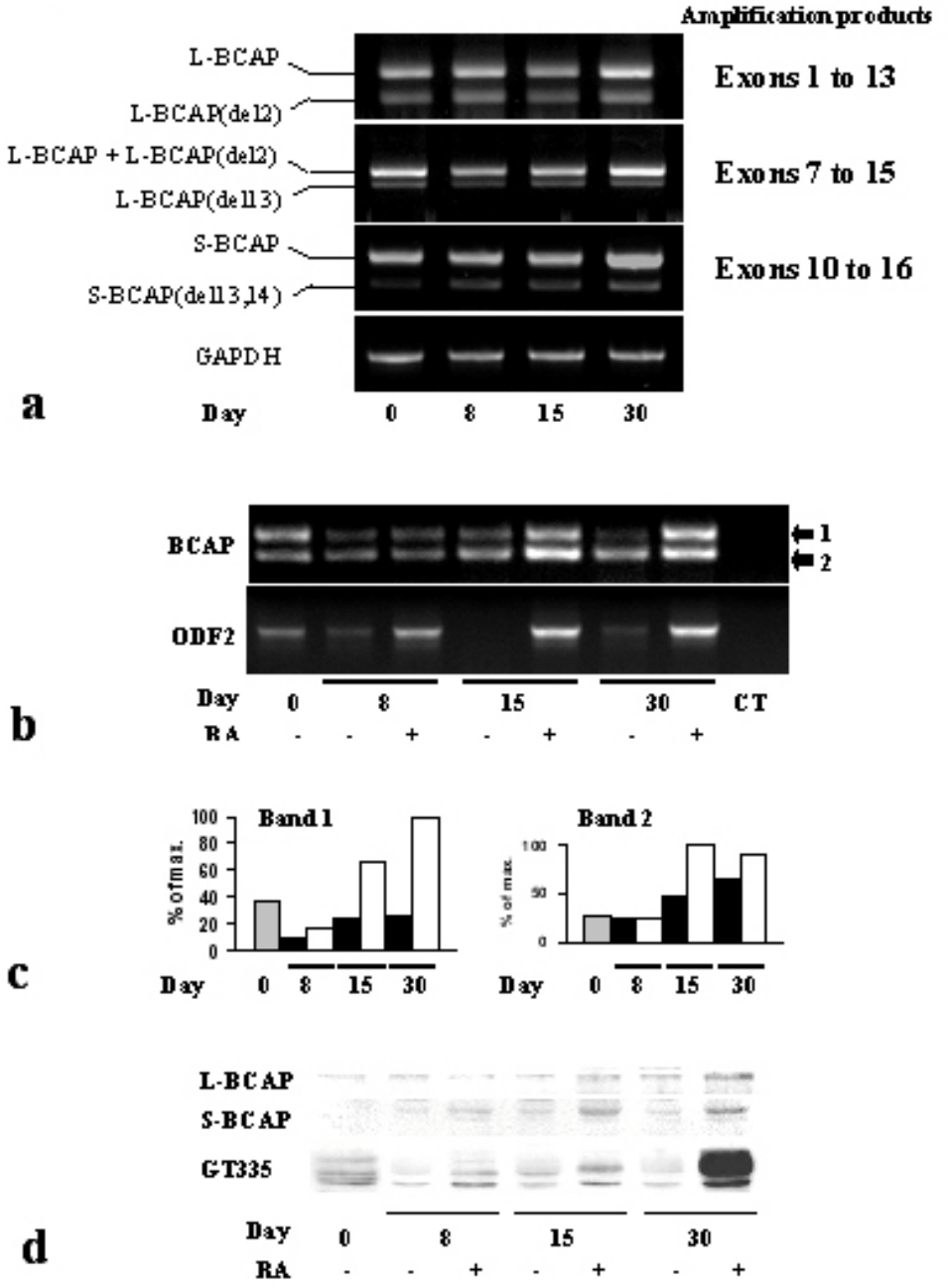

Figure 3. BCAP is highly enriched in differentiated cells. Total RNA (A, B) and proteins (D) were isolated at different times (0, 8, 15, 30 days) from HNE cells in ALI culture in the presence or in the absence of retinoic acid (RA). (A) RT-PCR analyses of BCAP isoforms during MCD (+RA) is performed using several sets of primers: L-BCAPs are amplified using primers localized in exons 1 (forward) and 13 (reverse) and in exons 7 (forward) and 15 (reverse), whereas S-BCAPs are amplified using primers localized in exons 9 (forward) and 14 (reverse) and in exons 10 (forward) and 15 (reverse). All L-BCAPs and S-BCAPs are expressed during *in vitro* MCD. GAPDH was used as a constant marker. (B) Semi-quantitative RT-PCR analyses of BCAP and ODF2 expression during MCD (+RA) or epidermoid differentiation (-RA). BCAP and ODF2 are mainly expressed in the presence of RA, and two mRNA are amplified. Control amplifications with no reverse transcriptase does not yield to any products. (C) RT-PCR data normalization by GAPDH. Left: BCAP mRNA expression corresponding to the upper band (S-BCAP). Right: BCAP mRNA expression corresponding to the lower band (L-BCAP, L-BCAP(del13), L-BCAP(del2) and S-BCAP(del13,14)). Gray: confluence day. White: MCD. Black: epidermoid differentiation. (D) BCAP expression in HNE cells using a specific BCAP pAb antiserum detected by western blotting. GT335, a mAb that detects polyglutamylated tubulins, gives the relative proportion of ciliated cells (Million et al., 1999). S- and L-BCAP are detected early during differentiation and the protein expression level increases during MCD (+RA). 10 µg of total protein extracts are deposited per lane.

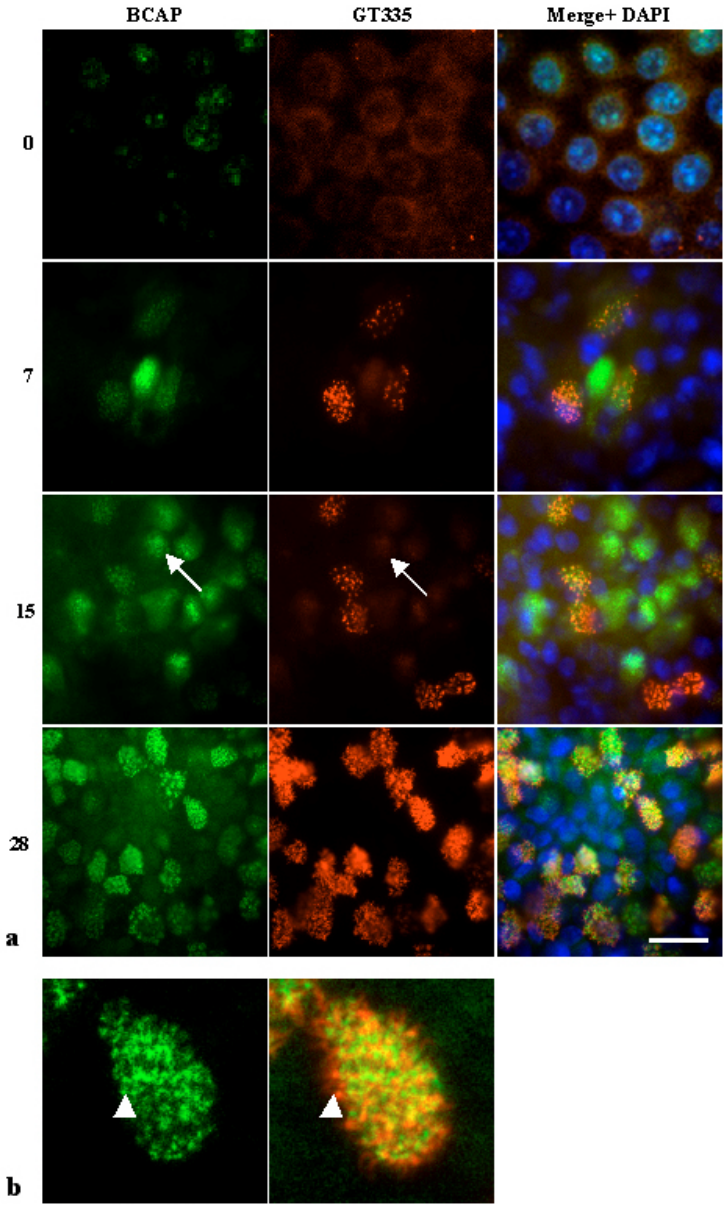

Figure 4. BCAP protein is enriched within basal bodies in HNE cells. (A) HNE cells in ALI culture are fixed during the time-course of MCD (0, 7, 15, 28 days) in the presence of retinoic acid, and processed for IF staining. Double-labelling experiments are achieved with anti-BCAP pAb (green) and GT335 mAb (red). Cytoplasmic staining is observed at cell confluence (day 0), while granular staining is detected in ciliated cells. BCAP does not colocalize with GT335 staining along cilia, but decorates basal bodies. BCAP detection appeares before GT335 labeling (arrow). Representative data from at least three independent experiments are shown. Basal or secretory cells are not labeled by anti-BCAP antiserum. Scale bar= 26 µm. (B) Higher magnification of ciliated cells. BCAP protein is localized within basal bodies and does not extend along the axonemes (arrow head).

respiratory tract infections that occur in patients with the autosomal recessive inherited disease, primary ciliary dyskinesia (PCD) (21).

In this study, we undertook a cDNA differential display analysis to identify new genes overexpressed during MCD of HNE cells. Genes have been listed by their function and several groups are probably not directly implicated in MCD, i.e. housekeeping genes, genes implicated in cell cycle control or cell death which are probably modulated in response to cell cycle arrest occurring during MCD or cell death resulting from rotary shaking, and genes implicated in transcription regulation and mRNA maturation which might reflect differential

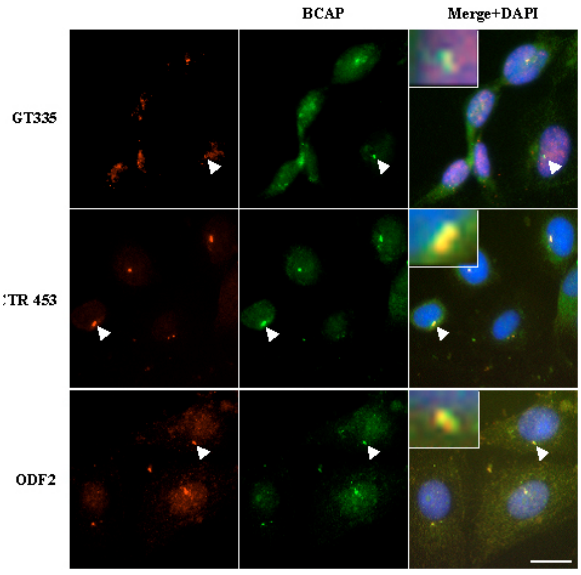

Figure 5. BCAP localizes to centrioles of 16HBE proliferative cells. Double-staining in IF using anti-centrosome Abs (CTR453, GT335, ODF2 mAb) and anti-BCAP pAb showing localization of BCAPs at the centrosome. Arrow heads indicates localization of the centrosome enlarged for each double-labeling. Representative results from three independent experiments are shown. Scale bar= 16 µm

gene regulation during MCD. One of them, ELF3, has been described as an epithelial-specific transcription factor (22) and may regulate genes implicated in MCD of epithelial cells. The last three gene groups (cytoskeleton, immunity/ inflammation, diverse) are probably relevant in MCD. For example, hp28 encodes a dynein light chain localized within the axoneme and its upregulation during MCD is relevant to cilia function. It was previously identified in another differential screening selecting genes downregulated upon IL-13 treatment, an inflammatory cytokine, which inhibits ciliated cell differentiation (23). The group of immunity/inflammation genes is of importance when we consider that the airway epithelium is the first target for inhaled pathogens and is the site of an immune response. In the sixth group, we have listed diverse genes found in our differential display, including genes associated with differentiation of other tissues (MAC-MARCKS, (24), NIF3L1BP1, (25)) or modulated upon cell differentiation (PLAC8, (26)). Therefore, we hypothesize that these genes are also related to MCD.

Among genes upregulated during MCD, we identified new protein isoforms encoded by one gene, *bcap* (for basal body and centriole associated protein), sharing homology with ODF2, a widespread scaffold component of the centrosome (7) and a microtubule-associated protein (8).

We showed that *bcap* is composed of 18 exons localized on the 1p22 locus. Its expression is regulated by alternative splicing and probably by the use of an alternative promoter. Indeed, 5 different BCAP spliced forms were identified. These forms were classified into two groups: long BCAP forms containing 15 (L-BCAP(del13) and L-BCAP(del2) or 16 exons (L-BCAP) and short BCAP forms containing 12 (S-BCAP) or 10 exons (S-BCAP(del13,14)). This result was in agreement with the detection of two mRNAs in Northern blot analyses (see Figure 2), and by amplification of two cDNAs by RT-PCR in HNE cells. These different forms could therefore be correlated with different functions.

BCAPs contained a large coiled-coil domain and showed homology with several coiled–coil proteins associated with the cytoskeleton: CEP63, CEP110 and CEP290, three proteins identified by a proteomic approach undertaken to characterize centrosome components (4), CLIP190, a MAP binding unconventional myosin (27), Nek2, a protein kinase regulating the centrosome structure (28), Nuf1/SPC110, a structural component of the spindle pole body (29), the human homologue of which is implicated in microtubule nucleation (30), and rootletin (CROCC), a component of ciliary rootlet (31). These homologies suggested that BCAP isoforms encoded new proteins involved in microtubule organization and ciliated cell differentiation. Sequence analyses of BCAP proteins also suggested the existence of a predicted ATPase domain homologous to the domain found in SMC ATPases (Structural Maintenance of Chromosome ATPases), implicated in cell division and chromosome partitioning, conserved in all organisms (20). It is noteworthy that two centriolar proteins sharing homologies with BCAP, Nuf1/SPC110 and rootletin also displayed a predicted SMC ATPase domain. The existence of such a domain suggested a role for BCAP in protein transport and in centriole migration to the apical cell membrane.

Tissue distribution of BCAP assessed by Northern blot clearly showed that BCAPs were mainly expressed in adult human trachea and testis, two tissues containing cells displaying motile axonemal structures. In contrast, BCAP mRNA was only weakly detected in human brain. Nevertheless, the BCAP mouse homologue was recognized by anti-BCAP pAb in ependymal cells cultivated *in vitro* (data not shown) and ependymal cells of the subventricular zone (SVZ) are also multiciliated cells (32). This discrepancy is likely due to the low proportion of ependymal cells among different cell types of brain. Indeed, Conversely, BCAP was weakly detected in non-ciliated tissues such as stomach and suprarenal glands, and by RT-PCR in non-ciliated cells (data not shown). We have shown that BCAP protein localization was not restricted to basal bodies, but was also associated with the centrosome of proliferating cells. Therefore, BCAP mRNA detected in non-ciliated tissues could correspond to the centrosomal association of BCAP. L-BCAP and S-BCAP were differentially expressed in ciliated cell-containing tissues and non-ciliated ones: both L- and S-BCAP mRNA were detected in trachea, whereas only S-BCAP or L-BCAP mRNA were detected in testis or brain, respectively.

BCAP was localized at basal bodies and the apical region of polarized epithelial cells, but was not

found along cilia axonemes. These results suggested that BCAP could act early during ciliated cell differentiation, basal body assembly, migration or anchorage to the apical cell plasma membrane. Indeed, BCAP was detected by confocal microscopy analyses in non-ciliated (GT335-negative) cells (33). Moreover, the mouse BCAP homologue was also localized within basal bodies of *in vitro* differentiated ependymal cells (data not shown). However, BCAP is probably not implicated in intraflagellar transport (IFT) as it was not detected along the ciliary axonemes. Using GT335, CTR453 and ODF2 mAbs as centriolar markers, we showed that BCAPs were colocalized with both centrioles in the Human Bronchial Epithelial cell line 16HBE. This result was in agreement with the low detection of BCAP mRNA in non-ciliated tissues. Therefore, BCAP localization could be involved in centrosomal function.

Thus, BCAP was localized at both centrioles in proliferative cells and basal bodies in human ciliated epithelial cells. BCAP temporal and spatial expression during differentiation suggested a putative role for this new protein in basal body formation and function. Identification of proteins that interact with different BCAP isoforms, co-immunolocalization studies at the EM level and RNAi in mouse ependymal cells should help to elucidate their functions. Moreover, regulation of L- and S-BCAPs in different cell types of a given tissue might reflect a specific function for each isoform.

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Abreviations: PCD: Primary Cilia Dyskinesis, HNE: Human Nasal Epithelial, MCD: MucoCiliary Differentiation, RA: retinoic acid, ALI: Air-liquid interface

Key Words: BCAP, Centriole, Basal Body, Human Nasal Epithelial Cells, Ciliated Differentiation

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