Molecular Cloning and Characterization of spurt, a Human Novel Gene That Is Retinoic Acid-inducible and Encodes a Secretory Protein Specific in Upper Respiratory Tracts*

Received for publication, October 15, 2002
Published, JBC Papers in Press, October 29, 2002, DOI 10.1074/jbc.M210523200

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Retinoids, such as all-trans-retinoic acid, play an essential role in the regulation of airway epithelial cell growth, differentiation, and gene expression. Using cDNA microarray, we identified a clone, DD4, that contains the cDNA of a novel gene, spurt (secretory protein in upper respiratory tracts) that was significantly induced by all-trans-retinoic acid in primary cultured human tracheobronchial epithelia. Two alternatively spliced spurt transcripts of 1090 and 1035 base pairs exist that contain the same open reading frame expressing a 256-amino acid peptide. The full-length spurt cDNA sequence spans a genomic DNA fragment of 7,313 bp, and the gene is located on chromosome 20q11.21. spurt mRNA is expressed at high levels in human nasal, tracheal, and lung tissues. In situ hybridization demonstrated that spurt message is often present in secretory cell types. The human spurt gene product is a secretory protein that contains a distinct signal peptide sequence in its first 19 amino acids. Mono-specific antibodies were generated to characterize spurt expression. Our data demonstrate that spurt is secreted onto the apical side of primary human airway epithelial cultures and is present in clinical sputum samples. spurt gene expression is higher in sputum and tissue samples obtained from patients with chronic obstructive lung disease. Our results provide the cloning and characterization of this tissue-specific novel gene and its possible relationship with airway diseases.

The mucociliary functions of conducting airway epithelia play an essential role in pulmonary defense against various inhaled pollutants. It has been reported that basal, ciliated, and secretory cells form a coordinated airway defense mechanism against inhaled air pollutants (1). Goblet cells in the airway epithelium and secretory cells in the submucosal glands are the major contributors to mucus secretion (2, 3). The secretory products contributed by each cell type are currently unknown. Therefore, identifying secretory products for each cell type allows for a marker to either assess the functional role of a specific cell type in maintaining homeostasis of mucociliary function or evaluate its role in the pathogenesis of various airway diseases.

Airway epithelial secretory proteins are divided into two major classes: mucins and non-mucins. Mucins are major secretory components that play an essential role in protecting and lubricating the airway epithelial surface. Mucins constitute a very large molecular weight glycoprotein family that are poly-dispersed and heavily O-glycosylated (4, 5). Currently, there are at least 12 mucin genes identified. MUC1, MUC2, MUC4, MUC5AC, and MUC5B are expressed at relatively high levels in the human respiratory tracts compared with other mucin genes (6–10). Non-mucin secretory proteins include lysozyme, lactoferrin, peroxidase, and antileukoprotease. These proteins contribute directly or indirectly to the pulmonary defense mechanism against infectious pathogens (12, 13). The cell of origin for most of these non-mucin secretory proteins is the serous cell contained within submucosal glands (11–13). However, there is still a large gap of information about the nature of these secretory proteins and their role in pulmonary defense.

Vitamin A and its metabolites, retinooids, are critical to the maintenance of mucociliary functions and secretions in the airway epithelium. In vitamin A-depleted animals, the airway epithelial surface changes from a mucociliary cell layer to a protective, squamous cell surface. This alteration can be reversed upon the addition of retinoids, such as all-trans-retinoic acid (ATRA) (14). Similar changes can be demonstrated in vitro in primary cultures of airway epithelial cells derived from humans and other species (15–17). Despite efforts by researchers, effects of retinoids on airway epithelia are not completely understood. We propose that retinoids induce differentiation through sequential changes in gene expression. This is based on observations that retinoid administration coincides with airway cell differentiation.

To test this hypothesis, we employed a two-color-based differential hybridization on a high density cDNA microarray membrane, which contained 30,000 cDNA spots (18, 19). These cDNA clones were individually selected from a pool of cDNA libraries created from human tracheobronchial epithelial

* This work was supported in part by National Institutes of Health Grants HL35635, ES09701, ES06230, 5F32HL09573, and HL04404, American Lung Association Grant RG-025L, and California Tobacco-Grants HL35635, ES09701, ES06230 5F32HL09573, and HL04404), © 2003 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in U.S.A.
Cloning of an ATRA-inducible spurt Gene in Human Airway

(TBE) cells. The membrane was differentially hybridized with cDNA probes derived from ATRA-treated and -depleted cultures of primary human TBE cells. Several ATRA-responsive cDNA clones were identified, and one of them, the DD4 clone, encoded the cDNA sequence of a novel gene. In this report, we characterize the expression of this novel gene in airway epithelia. Results from our studies suggest that the DD4 clone encodes a protein product that may be secreted by a secretory protein specifically produced by epithelial cells of the upper respiratory tract. Based on the characteristics of this gene, we subsequently named this gene spurt (secretory protein in upper respiratory tract).

MATERIALS AND METHODS

Sources of Human Airway Tissue and Cells— Human nasal, tracheobronchial, and lung tissues were obtained from the University of California at Davis Medical Center or from the Anatomic Gift Foundation (Laurel, MD) with consent. The Human Subject Review Committee of the University of California at Davis approved all procedures involved in tissue procurement. Excised tissues were transported to the laboratory in an ice-cold, minimal essential medium (Sigma). Primary human TBE cells were isolated from these tissues by a protease dissociation procedure and cultured in a serum-free hormone-supplemented medium as previously described (1, 17). Normally, the cells were grown in Dulbecco modified Eagle’s medium supplemented with six hormonal supplements as described previously (1, 17). Under this culture condition in the completed medium containing both the hormonal supplements and ATRA, under this culture condition in the completed medium containing both the hormonal supplements and ATRA, the human TBE cells differentiated into a mucociliary epithelium resembling that seen in vivo. The cDNA clones were packaged using a PBK CMV phagemid packaging system (Stratagene, La Jolla, CA).

From the 30,000 cDNA clones, we developed a high density microarray membrane (3.1 × 4.6 cm, Nitran N+ nylon membrane; Schleicher & Schuell, Keene, NH). This 30,000 cDNA microarray high density membrane was hybridized simultaneously with two-color cDNA probes derived from ATRA-treated (magenta) and untreated (cyan) cultures of primary human TBE cells as described before (18). Based on a quantitative ratio of cyan/magenta either greater than 5 or less than 0.2, clones, including our novel gene, were selected for further characterization.

In Situ Hybridization—cDNA clones obtained from the original phage library screening were converted to phagemids according to the manufacturer’s protocol (Stratagene). The recombinant plasmid was linearized with EcoRI or XhoI to generate antisense and sense templates, respectively. The linearized templates were in vitro transcribed with T7 and T3 RNA polymerases using MAXIScript™ according to the manufacturer’s recommendations (Ambion Inc., Austin, TX) to produce 35S-UTP-labeled antisense and sense cRNA probes, respectively. In situ hybridization was carried out as described previously (24–26).

Cloning and Sequencing of spurt cDNA—A human airway epithelial cell-specific cDNA library was screened to isolate spurt cDNA using a zAP cDNA library that was custom-made in our laboratory. The cDNA library was derived from human primary cultures of airway epithelial cells grown under Bi-CG conditions in the presence of 30 nM ATRA for 3 weeks. DNA sequencing was carried out at the Institutional DNA Core Facility (Department of Plant Genetics, University of California at Davis) using the fluorescence-labeled automated sequencing approach and separated by the ABI Prism model 377 automated DNA sequencer (Applied BioSystems, Foster City, CA). Four sequence primers (DD4-SEP1, 5’-CGGTCATGAGGAGTTGGGA-3’; DD4-SEP2, 5’-CCGAGAGCC-TTGAAATGGG-3’; DD4-SEP3, 5’-CGTGGCCCTCCTGTCACTG-3’; and DD4-SEP4, 5’-TAGTTGAGGCCACATGTTAG-3’) were used as follows: DD4-SEP1, 5’-GACGTCACTGATTCCTGCCC-3’; DD4-SEP2, 5’-TCCAGAGACCC-TTGAATTGGG-3’; DD4-SEP3, 5’-CGTGGCCCTCCTGTCACTG-3’; and DD4-SEP4, 5’-TAGTTGAGGCCACATGTTAG-3’.

The sequence data were analyzed with both Geneworks (IntelliGenetics, Inc., Mountain View, CA) and Lasergene (DNASTAR Inc., Madison, WI) software packages as well as with the online GCG software package SeqWeb (GCC, Madison, WI). Sequence homology to published sequences in public data bases was analyzed and determined by the BLASTn or BLASTp programs at the National Center for Biotechnology Information through Internet services. 5’-Rapid Amplification of cDNA Ends—The sequence of the full-length cDNA was determined by 5’ extension of RNA from human TBE cells cultured under the Bi-CG condition with 30 nM ATRA, using an end-labeled antisense primer (5’-GGCTAA-CAGCCCCTGAGAAC-3’) and the dideoxynucleotide chain termination method according to the manufacturer’s recommendations (Promega, Madison, WI). A base pair ladder was sequenced in parallel with 5’ extension product Taqtrack™ sequencing kit (Promega). For 5’-rapid amplification of cDNA ends, the marathon kit (Clontech) was used to synthesize the first strand cDNA from mRNA (1 µg) isolated from primary cultures of human TBE cells, which were cultured under air-liquid interface culture conditions and 30 nM ATRA for 3 weeks. Semi-quantitative RT-PCR was performed to evaluate first strand cDNA synthesis. This was followed up by second strand DNA synthesis and adaptor ligation to terminal ends. PCR with nested primers was performed for product amplification. The PCR products were subcloned into the TA vector (Invitrogen, San Diego, CA) for cloning and DNA sequencing.

5’-Rapid Transcription-Translation of spurt Protein—spurt protein was transcribed and translated in vitro by combining the MAXIScript™ T7/T3 kit and Retic Lysate IVT kit (Ambion). Template DNA was obtained by subcloning the ORF fragment of spurt cDNA into a pcDNA3 expression vector (Invitrogen). 1 µg of DD4 (spurt) DNA template was then mixed with the corresponding RNA polymerase, nTPs, and transcription buffer, and the 20-µl reaction mixture was incubated for 45 min at 37 °C.

In vitro transcribed RNA (0.5 µg/reaction) was then labeled with 4 µl (1200 Ci/mmol) of [35S]methionine (ICN), reticulocyte lysate, and master mix containing 200 mm creatine phosphate, 3 m potassium acetate,
10 mM MgCl₂, and an amino acid mixture (2 mM) (Ambion) for 60 min at 30 °C. Adding 4× volume of 1 mg/ml RNase A and continuing the incubation at 30 °C for 10 min more terminated the translation reaction. Four volumes of Laemmli SDS sample buffer (2.5% SDS, 0.1 M Tris, pH 6.8, 10% glycerol, 350 mM 0.025% β-mercaptoethanol, 0.1% bromphenol blue) were added (27). The translated products were separated by electrophoresis in a 12% SDS-polyacrylamide gel. For imaging, dried gels were exposed to x-ray film.

Construction and Transfection of a spurt FLAG-tagged Fusion Protein—A fusion protein with FLAG attached to the N terminus of spurt protein was made to identify the size of spurt protein. For FLAG tagging, the entire coding region of the novel cDNA was fused in frame 3′ to a FLAG tag in the vector pFLAG CMV2 (Sigma). To prepare the FLAG-spurt expression vector, the ORF fragment of spurt was amplified by PCR using appropriate primers (forward Primer DD4FLAG-1F, 5′-AGAATTCCTCCAGGCATCCGATGTTAAGCC-3′; reverse primer DD4FLAG-2R, 5′-TGGTCTAGAGCCATGGGATGGTACAC-3′) containing EcoRI and BglII sites at the 5′- and 3′-ends, respectively. The PCR products were inserted into EcoRI/BglII sites of the pFLAG vector (Sigma). Modification was made to place a perfect Kozak sequence (5′-CCACCC-3′) (28) immediately upstream of the presumed translational start codon of the ORF of spurt. The vector-insert junction was verified by nucleotide sequencing. For transient transfection studies, 0.5 μg of FLAG-spurt plasmid was transfected into 5 × 10⁵ HBE cells in a 35 mm Petri dish with LipofectAMINE (Invitrogen). Two days post-transfection, one dish from each transfection group was harvested for protein isolation and Western blot analysis.

Polyclonal Antibody Production and Western Blot Analysis—A 16-mer oligopeptide antigen was synthesized (Research Genetics, Inc., Huntsville, AL) using the deduced amino acid sequence from Arg¹⁸⁸ to Cys³⁶ of spurt. The peptide was conjugated to multiple antigen peptide to increase its antigenicity, and rabbit-based polyclonal antibodies were generated as described before (29). The specificity of the polyclonal antisera was determined by enzyme-linked immunosorbent assay and Western blot analysis.

For Western blot analysis, cultured cells were harvested as described (30, 31). Supernatant protein concentrations were determined by the method (Bio-Rad). From the soluble portion of the sputum samples, 30 μg of total protein was used for SDS-PAGE and Western blotting.

Sputum Sample Preparation—Sputum samples were collected from subjects using a protocol approved by the University of California at Davis Institutional Human Subjects Review Board. Subjects were randomly asked to generate sputum during a routine visit to a general pulmonary clinic. Sputum generation was directly observed to ensure collection of an adequate specimen. The samples were discarded if the fluid viscosity was more consistent with saliva compared with sputum. All expectorated sputum samples were immediately stored on ice. During preparation, sputum samples were placed in a shaking water bath at 37 °C for 20 min to ensure complete homogenization and in the presence of a protease inhibitor mixture that included 1 μM iodoxacetamide, 25 μM mepropion, 10 μM leupeptin, 5 mM phenylmethylsulfonyl fluoride, and 10 mM dithiothreitol to reduce protein degradation and disulfide cross-links (Sigma). The samples were spun to separate gel matrix from the soluble phase (32, 33). The protein concentration of the soluble portion of the spume sample was determined by Lowry’s method (Bio-Rad). From the soluble portion of the sputum samples, 30 μg of total protein was used for SDS-PAGE and Western blotting.

RESULTS

Isolation of an ATRA-responsive Gene—A high density DNA microarray membrane containing 30,000 cDNA clones derived from cDNA libraries of human TBE cells was used to differentially screen for ATRA-responsive genes (18, 19). One of the ATRA-inducible novel genes, DD4, was selected for further studies. Northern blot hybridization with RNA isolated from primary human airway epithelial cultures treated with or without ATRA (30 nM) confirmed that the expression of DD4 is strongly induced by ATRA (Fig. 1A). The RNA staining gel (Fig. 1B) confirmed equal loading of the RNA samples and lack of any RNA degradation. To further confirm the ATRA-responsive nature of DD4, we examined DD4 induction at a variety of ATRA concentrations and times. Northern blot hybridization revealed that the DD4 message was strongly induced by ATRA in a dose-dependent manner (Fig. 2A). The induction could be seen at concentrations higher than 30 nM. DD4 gene expression was detected 7 days after starting retinoid treatment, which indicates that induction by retinoids is a late event (Fig. 2B).

In addition, cell culture conditions had an effect on DD4 mRNA expression levels. The highest induction of DD4 message was observed when cultured cells were maintained on collagen gel under air-liquid interface culture conditions (BI-CG). Other culture conditions, such as plating on CG substrate alone or with an air-liquid interface alone (B1), resulted in lower levels of DD4 gene expression. Expression was the lowest in cells plated on a TC plastic surface. Despite these changes in DD4 gene expression, quantitative results demonstrated that ATRA enhanced DD4 mRNA expression at least 10-fold in all culture conditions (Fig. 1C).

Expression Pattern of spurt mRNA in Normal Tissues—To identify the expression pattern of the DD4 gene in normal tissues, we employed a multiple-tissue Northern blot hybridization. Using a human tissue distribution membrane consisting of total RNA derived from various normal human tissues, Northern blot analysis revealed significant DD4 message levels in the trachea, bronchi, and lung. No hybridization signal was observed in RNA samples harvested from other tissues (Fig. 3). Similar results were obtained using the human DD4 probe against a murine multiple tissue distribution membrane, which included 14 different mouse tissues. DD4 expression in the mouse was observed only in the nasal septum, trachea, and lung (data not shown). In addition, nasal epithelial cells freshly obtained from human nasal turbinates and nasal polyps exhibited relative high DD4 expression levels by Northern blot analysis. These observations suggest that DD4 expression is specific to the conducting airways.

In situ hybridization demonstrated that DD4 mRNA is specifically expressed in the secretory ducts and submucosal glands of normal human tracheobronchial tissue sections (Fig. 4). For comparison, in situ hybridization with a sense cRNA probe did not display an observable signal when compared with
background (data not shown). In situ hybridization of tissue sections from “normal” patients showed low abundance DD4 message in the surface epithelium. Furthermore, the expression level of DD4 is highest in the trachea and decreases progressively from the proximal (bronchial region) to distal (bronchiolar region) airways in the human lung (Fig. 5). This pattern closely correlates with previous reports on serous cell distribution patterns in the respiratory tract (12, 34).

Expression Pattern of spurt mRNA in COPD Tissues—Importantly, although the gene expression of DD4 was generally confined to the submucosal gland region of normal human tracheobronchial tissues, we were able to detect elevated DD4 message in the airway surface epithelia in all tissue sections obtained from four patients with chronic obstructive pulmonary disease (COPD). For in situ hybridization, we labeled DD4 riboprobes by an enzymatic method, obtaining concurrent hybridization, we labeled in situ hybridization of tissue sections from normal level of message in the surface epithelium. Furthermore, the expression pattern of this gene.

To identify the transcription start site, 5'-end primer extension was performed (Fig. 7B). These results indicate that the transcription start site is at least 12 bp upstream of the longest cDNA clone, DD4-C4. We obtained similar results using Eukaryotic Neural Network Promoter Prediction (NNPP/Eukaryotic) software to theoretically predict the promoter region and transcription start site (data not shown). However, the smear pattern seen with our primer extension results suggested incomplete primer extension. We found that there were several consecutive guanine residues in the region of the transcription start site. This potentially will interfere with the capability of reverse transcriptase to fully extend to the upstream 5'-end of the actual starting site. In support of this, additional data from recently released EST clones (BG546713, BG570614, BG538707, and BG537983) suggest that the actual transcriptional starting site is five base pairs upstream of our original results.

Based on PCR data, we confirmed two alternatively spliced products of the DD4 gene (Fig. 7C). Using the same set of primers, we were able to amplify two products, 1090 and 1035 bp long, that differ by 55 bp at the 3'-untranslated end. Both amplified clones contain an open reading frame of 256 amino acid residues. For both products, the region adjacent to the translational initiation codon contains a favorable Kozak sequence (28). Both cDNA products contain the same polyadenylation signal, AUAATA, located 19 bp upstream of the polyadenylation site. Although the 55-bp size difference of the two transcripts is distinguishable using reverse transcription-PCR amplification methods, we are unable to resolve the two transcripts with Northern blot analysis.
B, the same serial tissue sections as in the submucosal gland region in the trachea of a COPD patient (100×). Compared with normal tissue sections (Fig. 5), was elevated in both the surface epithelium and the submucosal glands (200×). C, clearly demonstrates the positive staining of face epithelium (200×). Higher magnification epithelium or the submucosal glands (100×). Sense probe and did not show observable signal in either the surface epithelium or the submucosal glands (100×).

Characterization of Protein Expression—The peptide sequence analyses suggested that both DD4 cDNAs encode a protein with 256 amino acid residues. The predicted isoelectric point of DD4 is 5.497, and the calculated molecular mass is 26,711 daltons. To confirm the accuracy of the deduced amino acid sequence, we used in vitro transcription-coupling translation. This approach demonstrated a major translation product at 25 kDa in SDS-PAGE (Fig. 8A, lane 3). The slight discrepancy between the in vitro translated product and the predicted molecular mass of 26.7 kDa is probably due to the migration behavior of DD4 protein in gel. In addition, we performed transient transfection studies using a chimeric construct of pFLAG fused in frame to the coding sequence of the DD4 gene under the direction of a CMV promoter. Western blot analysis using anti-FLAG M5 antibody demonstrated a single protein product at the 27-kDa position. Mock (no transfection) and control transfections (control pFLAG vector with no cDNA insert) yielded no detectable signal (Fig. 8B).

The DD4 Gene Product Is a spurt Protein—To analyze the protein structure of DD4, several web-based protein structure analysis software programs were used. SignalP identifies secretory signal peptides and predicts the possible cleavage site of putative signal peptide sequences (35). Based on the analysis by this program of the deduced amino acid sequence, the human DD4 gene contains a distinct signal peptide sequence in its first 19 amino acids (data not shown). In addition, SignalP analysis predicted Ala19 to Gln20 as the most likely cleavage site. Another software program, PSORT, detects the sorting signals of proteins and predicts their subcellular locations (36). The results obtained from PSORT suggested a 33.3% probability that spurt is extracellularly secreted (data not shown). Both of these analyses strongly suggest that DD4 is a secretory protein.

Hydrophobicity analysis, based on the method of Kyte and
Cloning of an ATRA-inducible spurt Gene in Human Airway

Fig. 8. Successful DD4 in vitro transcription/translation and FLAG-tagged human DD4 protein expression. A, the complete DD4 cDNA was cloned into a pcDNA3 expression vector and translated in a reticulocyte cell lysate in the presence of [35S]methionine. The reaction products were analyzed by 12% (w/v) SDS-polyacrylamide gel electrophoresis, followed by autoradiography. The reaction was performed in the presence of capped XeF-1 RNA (lane 1, positive control) and in the absence of RNA (lane 2, negative control). The DD4 translated gene product was observed with an approximate molecular mass of 25 kDa as indicated (lane 3). B, human HBE1 cells were transiently co-transfected with either no DNA (lane 1), pFLAG CMV2 empty expression vector (lane 2), or pFLAG CMV2 expression vector inserted with the DD4 ORF (lane 3), and protein extracts were analyzed by Western hybridization as described above. The proteins were transferred to a polyvinylidene difluoride membrane and were incubated with the anti-FLAG antibody M5 at a 1:800 dilution. The arrow corresponds to a FLAG-tagged DD4 fusion protein.

Doolittle (59), predicted the hydrophilic/hydrophobic regions of DD4 (Fig. 9A). Based on this analysis, an oligopeptide that corresponds to a highly hydrophilic, highly antigenic region of 16 amino acids with high surface probability (Arg165–Cys180) was synthesized and used as an immunogen to generate polyclonal antibodies in rabbit. Using anti-DD4 antibody, DD4 protein was detected from cell culture supernatants collected in a reticulocyte cell lysate in the presence of [35S]methionine. The cDNA was cloned into a pcDNA3 expression vector and translated.

Fig. 9. Secretory nature of human DD4 is recognized by a polyclonal anti-DD4 antibody to DD4 protein expression in culture supernatants. A, schematic representation of the human DD4 (spurt) protein that includes a hydrophilicity plot (Kyte-Doolittle), an antigenic index (Jameson-Wolf), and a surface probability plot (Emini). Lasergene software was used to generate the analyses. The horizontal axis represents the corresponding amino acid position of DD4 (spurt), and the vertical axis of each plot represents arbitrary units. The amino acid residues that we used to generate anti-DD4 (spurt) polyclonal antibodies are marked in a shaded rectangular box, which corresponds to a region of high hydrophilicity and high surface probability (Arg165–Cys180). B, HBE1 cells and human primary TBE cells were treated with and without 3 × 10−7 M ATRA in air-liquid interface culture conditions. Supernatants from the apical region were collected and analyzed by Western hybridization using anti-DD4 (spurt) antibody. As indicated by a hybridization band at ~25 kDa (arrow), increased protein expression was observed in cell culture conditions that included ATRA.

Functional Analysis of spurt Protein—To elucidate the function of spurt protein, various bioinformatics search methods were performed, including InterPro Scan, ScanProsite, ProfileScan, pfam HMM search, and PATTINPROT. Using a Smith-Waterman algorithm, the putative spurt amino acid sequence has 27.3% homology to the C-terminal domain of the bactericidal/permeability increasing protein (BPI) that is located at chromosome 20q11.23 (37, 38) (Fig. 11A). Furthermore, when reverse position-specific BLAST (39) was performed to search against the Conserved Domain Data Base, spurt aligns to a conserved domain named BPI 1 that is derived from the N-terminal domain of BPI (SMART accession number SM0328) (40–42) (Fig. 11B). In addition, preliminary findings demonstrated a conserved phosphorylation site for casein kinase II from Ser190 to Asp193.
interface culture conditions (BI-CG). These data suggest that cells were plated on a collagen gel substratum with air-liquid differentiation limited to specialized cells in the human airway. In addition, these conditions, the differentiated features are not as extensive (1). Under these culture conditions, human airway epithelial cells develop into a fully differentiated cell layer with a collagen gel substratum, and retinoid supplements without and with collagen gel substrata. Optimized changes from tissue culture plates alone (TC) to biphasic conditions without and with collagen gel substrata. The function and expression of the spurt gene is currently unknown.

In this study, we utilized a microarray assay to identify an ATRA-inducible, novel secretory protein, spurt, which is secreted by conducting airway epithelia. Our results indicate that there is alternative splicing of spurt in the 9th exon that generates two transcripts with the same ORF. How this alternative splicing affects the function and expression of the spurt gene is currently unknown.

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duction by retinoids. spurt was originally identified in our microarray study as an ATRA-responsive gene. This induction was further confirmed by Northern blot analyses as shown in our data. However, Bingle’s research group described high levels of plunc RNA expression in cultured airway epithelial cells without any retinoid treatment and showed that this expression was not altered by retinoid treatment as indicated in the manuscript. Although we observed higher expression levels of spurt mRNA in human nasal turbinates and trachea, we did not identify a significant amount of spurt RNA expression in primary cultures of normal nasal epithelial or tracheobronchial epithelial cells under retinoid-depleted culture conditions. In addition, we did not demonstrate the expression of spurt or plunc-like message in NCI-H647 cell lines obtained from the ATCC. This cell line was shown by Bingle’s group to be the only cell line constitutively expressing plunc message. The reason for these discrepancies is currently unclear.

Based on our protein structural analyses of spurt, two functional motifs have been identified. One of them is a conserved phosphorylation site from Ser190 to Asp193 for casein kinase II, phosphorylated. However, based on the similarity of the molecular weight obtained from amino acid sequence estimations and the mobility of spurt in SDS-PAGE, we concluded that phosphorylation is less likely.

The second ‘putative’ domain identified in spurt shows amino acid sequence homology to both the N-terminal and C-terminal domains of BPI. BPI has been implicated in bactericidal processes (53–57). The presence of a BPI-like domain in spurt protein suggests an antibacterial role for this novel protein. Consistent with this idea, our in situ hybridization studies demonstrated that spurt is frequently expressed in the serous cells of secretory ducts and submucosal glands. Some serous cell secretions, such as lysozyme, are known to have antibacterial activity. It is of interest to note that plunc, a spurt-like mouse gene, has been shown to be expressed in mouse thymus, a tissue that harbors antimicrobial gene products (58). These observations suggest that spurt and plunc represent members of a new bactericidal gene family.

To better understand the role of spurt in human disease, we compared the level of spurt expression between normal subjects and subjects with chronic lung diseases. Based on in situ hybridization, we observed a higher level of spurt mRNA expression in submucosal gland serous cells of patients with COPD. In addition, the submucosal gland ducts and airway epithelial surface of lung tissue from COPD patients also displayed expression of spurt mRNA. We were unable to demonstrate spurt mRNA expression in the airway epithelial surface of lung tissue obtained from normal patients. Similarly, Western blot analysis demonstrated that spurt protein expression is significantly elevated in all sputum samples obtained from COPD patients compared with normal subjects. We postulate that the recurrent infections seen in patients with chronic lung diseases such as emphysema and chronic bronchitis results in the up-regulation of airway bactericidal proteins such as spurt. This higher expression of spurt involves both increased induction of the gene in submucosal gland serous cells that normally express the gene as well as the induction of cells in the submucosal gland ducts and airway surface epithelia that normally do not express spurt.

In conclusion, we have shown that spurt is a novel upper airway-specific secretory protein that is induced by retinoids. Two alternatively spliced forms of spurt exist. spurt has putative antibacterial functions, and the expression of this protein is increased in lung diseases such as chronic bronchitis and emphysema. The mechanisms and purpose of this up-regulation are actively being investigated in our laboratory.

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*J. Biol. Chem.* 2003, 278:1165-1173.
doi: 10.1074/jbc.M210523200 originally published online October 29, 2002

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