The emergence of a highly transmissible lineage of *cbl*$^+$ *Pseudomonas (Burkholderia) cepacia* causing CF centre epidemics in North America and Britain

LI SUN[1], RU-ZHANG JIANG[1], SUZANNE STEINBACH[2], ALISON HOLMES[1], CRAIG CAMPANELLI[1], JANET FORSTNER[3], UMA SAJJAN[3], YING TAN[4], MARGARET RILEY[4] & RICHARD GOLDSTEIN[1]

[1]*Section of Molecular Genetics, Maxwell Finland Laboratory for Infectious Diseases, Boston University School of Medicine and Boston City Hospital, 774 Albany Street, Boston, Massachusetts 02118, USA*
[2]*Department of Pediatrics, Boston University School of Medicine and Boston City Hospital, Boston, Massachusetts 02118, USA*
[3]*Research Institute, Division of Biochemistry, The Hospital for Sick Children, Toronto, Ontario, M5G 1X8, Canada*
[4]*Department of Biology, Yale University, New Haven, Connecticut 06511, USA*
Correspondence should be addressed to R.G.

The rapid increase in *Pseudomonas (Burkholderia) cepacia* infection in cystic fibrosis (CF) patients suggests epidemic transmission, but the degree of transmissibility remains controversial as conflicting conclusions have been drawn from studies at different CF centres. This report provides the first DNA sequence-based documentation of a divergent evolutionary lineage of *P. cepacia* associated with CF centre epidemics in North America (Toronto) and Europe (Edinburgh). The involved epidemic clone encoded and expressed novel cable (Cbl) pili that bind to CF mucin. The sequence of the *cblA* pilin subunit gene carried by the epidemic isolates proved to be invariant. Although it remains to be determined how many distinct, highly transmissible lineages exist, our results provide both a DNA sequence and chromosomal fingerprint that can be used to screen for one such particularly infectious, transatlantic clone.

Pseudomonas aeruginosa accounts for up to 90% of morbidity and mortality in patients with cystic fibrosis (CF) following persistent infection over a period of years. However, during the last decade, as many as 40% of the patients in some CF centres[1–4] have also become infected with *Pseudomonas (Burkholderia) cepacia*. About 20% of the latter die from bacteremia, or aggressive pulmonary infection over a few months[5,6]. While the significant increase in *P. cepacia* infection suggests epidemic spread[3,6–8], the source and transmissibility of *P. cepacia* remains controversial[9]. Nonetheless, given the potentially grave consequence of *P. cepacia* infection, stringent infection control policies have been adopted, many CF camps in North America have been closed and all but one lung transplant centre have ceased to accept *P. cepacia*-infected CF patients as transplant candidates.

The epidemiology of *P. cepacia* infection has been examined by both ribotyping[1,9] and pulsed-field gel electrophoresis (PFGE)-based resolution of chromosomal macro-restriction fragment length polymorphisms (RFLPs)[3,9,10]. Comprehensive studies applying both methods generated two very different conclusions regarding clonality, persistence, and transmissibility. One study in the United Kingdom (Western General Hospital, Edinburgh), found a clonal relationship among isolates from 13 patients over six years[3]. In contrast, during an eight-year period at a US CF centre (University of North Carolina (UNC) Hospitals, Chapel Hill), not a single identical or closely related strain was found among 23 infected clinic and lung transplant patients[9]. Serial isolate analysis further confirmed this picture, typically demonstrating persistent infection by a single strain.

There also existed an isolate collection from another CF centre (Hospital for Sick Children, Toronto), where there was anecdotal evidence for an epidemic of *P. cepacia*. Although the isolates were not characterized for genetic relatedness, they had been uniformly resolved to express peritricious, giant cable (Cbl)-like pili that specifically bound to CF mucin and airway epithelial cells[4,11–13]. As the *cblA* pilin subunit gene encoded by all 15 of the Toronto isolates was the first such gene characterized for *P. cepacia*, a subsequent hybridization-based survey for the presence of *cblA* was carried out on multiple isolates from eight other CF centres in the United States and Europe as well as clinical and environmental strains. All of these isolates were *cblA*$^-$ except for one isolate from a CF centre in Jackson, Mississippi.

Studies described in this article examine the genetic relatedness of *cblA*$^+$ and *cblA*$^-$ strains. The evolutionary picture generated indicates the emergence of a highly transmissible lineage, seemingly adapted for efficient transmission in the CF population. The resolved genetic markers uniquely associated with this lineage may be used to rapidly identify its presence and are therefore of immediate practical importance to CF centres in both Europe and North America.

Genetic relatedness of *P. cepacia* isolates

The observations described above led us to characterize the epidemiological relatedness of the 15 Toronto isolates expressing mucin-binding Cbl pili. To investigate the genetic relationship between these isolates and those found elsewhere, we included clinical and environmental isolates as well as 78 strains from the

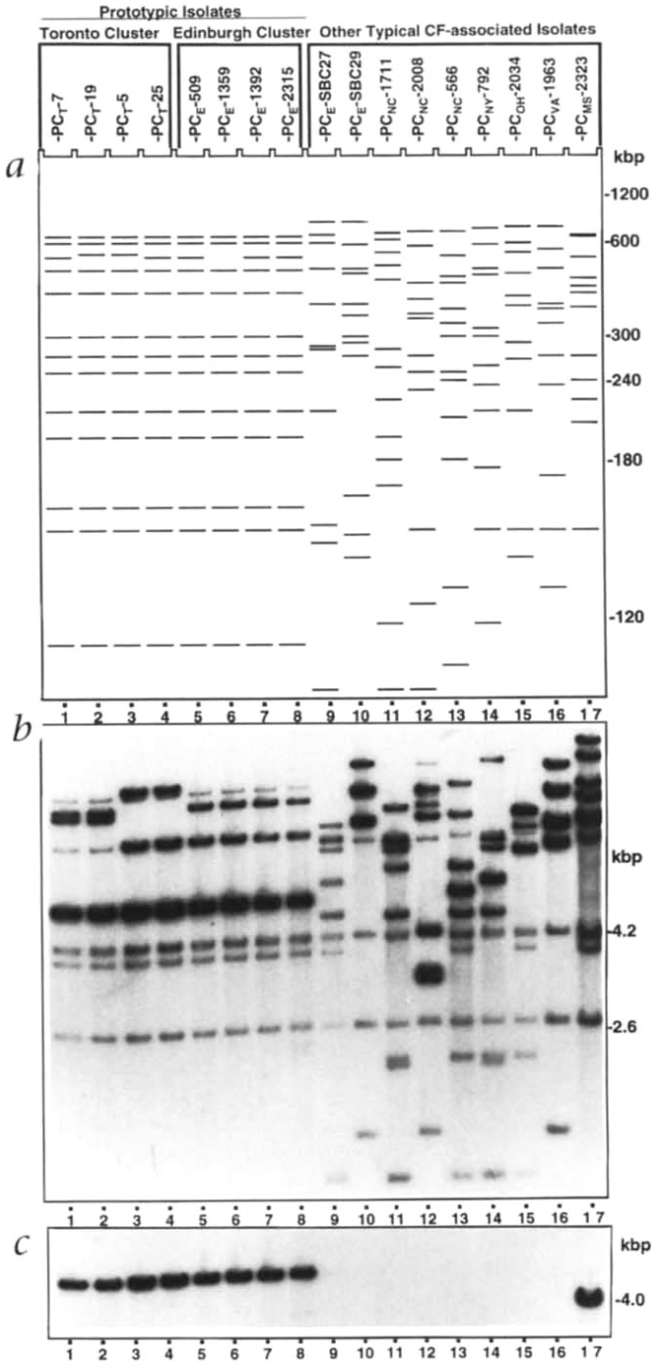

Fig. 1 RFLPs of 17 *P. cepacia* isolates cited in the text and Methods sections. Lane order for the 17 isolates is maintained in the three parts of the figure. Isolate numbers of examined strains appear at the top of the figure immediately above each lane. Subscript letters preceding isolate number indicate CF centre from which *P. cepacia* (PC) strain was isolated: PC_E, Edinburgh, Scotland; PC_{MS}, Jackson, Mississippi; PC_{NC}, Chapel Hill, North Carolina; PC_{NY}, New York, New York; PC_{OH}, Cleveland, Ohio; PC_T, Toronto, Canada; and PC_{VA}, Norfolk, Virginia. *a*, PFGE-resolved, chromosomal *Spe*I macro-RFLPs. As described previously[9], samples were prepared and restriction fragments separated by pulsed-field gel electrophoresis with a CHEF Mapper system (Bio-Rad) through 1% agarose using a field strength of 6 V/cm and an initial and final pulse time of 1.2 s and 54 s, respectively. Fragment sizes were determined using a λ concatenate ladder (not shown). Bar-code format translation of chromosomal fingerprint profiles was made using a Macintosh Quadra 950 running Gene Construction Kit (Texto). Fragments below 100 kbp are not shown. In the latter range, Toronto and Edinburgh isolates displayed in lanes 1–8 had two identical fragments (60 kbp and 48 kbp). Other isolates (lanes 9–17) had polymorphic sets of three to six fragments in this lower range. *b*, *rrn* (ribosomal RNA operon) *Eco*RI RFLPs. Southern blot hybridization methods were as we described previously[9,26] using a ^{32}P-labelled *rrnB* probe spanning the entire *rrnB* operon of *E. coli* K12. *c*, *cblA* hybridization analysis of *Eco*RIgenerated RFLPs. This was accomplished by stripping bound *rrn* probe from the membrane used in Fig. 1*b* followed by hybridization with a previously described *cblA* gene probe[4] using standard methods[26,27].

seven other CF centres cited above that were *cblA*$^-$. At this time the report of RFLP-identical *P. cepacia* isolates transmitted among patients at an Edinburgh CF centre appeared, and we obtained the involved strains[3] to include in this phylogenetic characterization (Fig. 1*a*, *b*). Profiles in lanes 9–17 of both *a* and *b* of Fig. 1 depict typical polymorphic patterns resolved for isolates from different CF centres. For these isolates mean D (Dice coefficient of similarity)[14] for any pair by PFGE-resolved chromosomal macro-RFLP profile was 0.14 ± 0.07 (Fig. 1*a*), a level of diversity not significantly different from that found previously among eight independently isolated American Type Culture Collection (ATCC) environmental and clinical control isolates[9]. A similar degree of chromosomal RFLP variability was found between the other CF-associated isolates from the seven CF centres (results not shown), confirming that these are epidemiologically distinct strains with RFLP variability not significantly different from that of the random collection of ATCC strains ($0.1 > P > 0.05$). Despite the lower discriminatory power of ribotyping[9], a similar degree of phylogenetic relationship among these CF-associated isolates is apparent in Fig. 1*b*.

The heterogeneity of the RFLP profiles of the isolates from the seven CF centres (for example, lanes 9–17, Fig. 1*a*, *b*) is similar to that described in a previous study involving multiple isolates from 23 patients at the UNC Hospitals CF centre[9]. This degree of variability contrasts markedly with the two closely related, conserved RFLP patterns found for the 15 *cblA*-encoding Toronto CF centre isolates (lanes 1–4, Fig. 1*a*, *b*). Here, by examining both PFGE and ribotype RFLP profiles, the coefficient of similarity among the Toronto isolates proved to be very high, with PFGE D = 0.95 ± 0.03 and ribosomal RNA operons (*rrn*) D = 0.87 ± 0.09. This contrasted with *(a)* the mean D value among isolates from the other seven CF centres, which was very low (for example, lanes 9–17, Fig. 1*a*, *b*), and *(b)* the mean D between the Toronto isolates and the other CF centre isolates, which was also very low: PFGE D = 0.20 ± 0.07, *rrn* D = 0.39 ± 0.09. These findings strongly suggest that all 15 of the Toronto CF centre isolates were members of a unique lineage associated with an epidemic.

Displayed in lanes 5–8 of Fig. 1*a* and *b* are *P. cepacia* PFGE and ribotype RFLP profiles of isolates from CF patients at the Edinburgh CF centre[3]. The RFLP profiles displayed in Fig. 1*a* and *b* also indicate the presence of an epidemic clone, as D for any analysed pair by either type of RFLP profile was very high (PFGE D = 0.98 ± 0.02, *rrn* D = 1.0). Further, pairwise comparison of the Edinburgh strains to the closely related Toronto CF centre strains (lanes 1–4 of Fig. 1*a*, *b*) likewise produces robust D values (PFGE D = 0.97 ± 0.03, *rrn* D = 0.90 ± 0.04), strongly suggesting that the

Edinburgh and Toronto isolates are members of the same unique lineage despite the Atlantic Ocean barrier.

Phylogenetic relationships of *P. cepacia* isolates

Based on ribosomal RNA operon (*rrn*) RFLP profiles, phylogenetic relationships of the 133 isolates described above and in the Methods section were determined with the neighbour-joining method[15]. Confidence intervals on the tree topology were estimated by bootstrapping analysis[16] (Fig. 2). The resultant phylogenetic tree indicates that the cluster of Toronto and Edinburgh isolates comprises a single, clonally related lineage. The remaining, independently isolated strains from other CF centres are as distantly related to one another as they are to either the Toronto/Edinburgh clusters or the independently isolated non-CF clinical and environmental strains.

Cbl phenotype and genotype of Edinburgh CF isolates

We then examined the epidemic Edinburgh isolates to see if, as do the Toronto strains[4], they expressed Cbl pili and encoded the *cblA* gene. Phenotypic survey using electron microscopy revealed that these highly transmissible strains expressed appendage pili that were structurally equivalent to those expressed by all of the *cblA*$^+$ Toronto isolates[4,13] (Fig. 3). Genotypic survey was carried out by stripping *rrn*-probe from an *Eco*RI chromosomal digest membrane (Fig. 1b) followed by hybridization with a *cblA* probe. The highly transmissible Edinburgh isolates as well as the closely related Toronto clones encode *cblA* (Fig. 1*c*, lanes 1–8).

cblA gene sequence-based test of clonality

Because the implications of our studies have the potential to in-

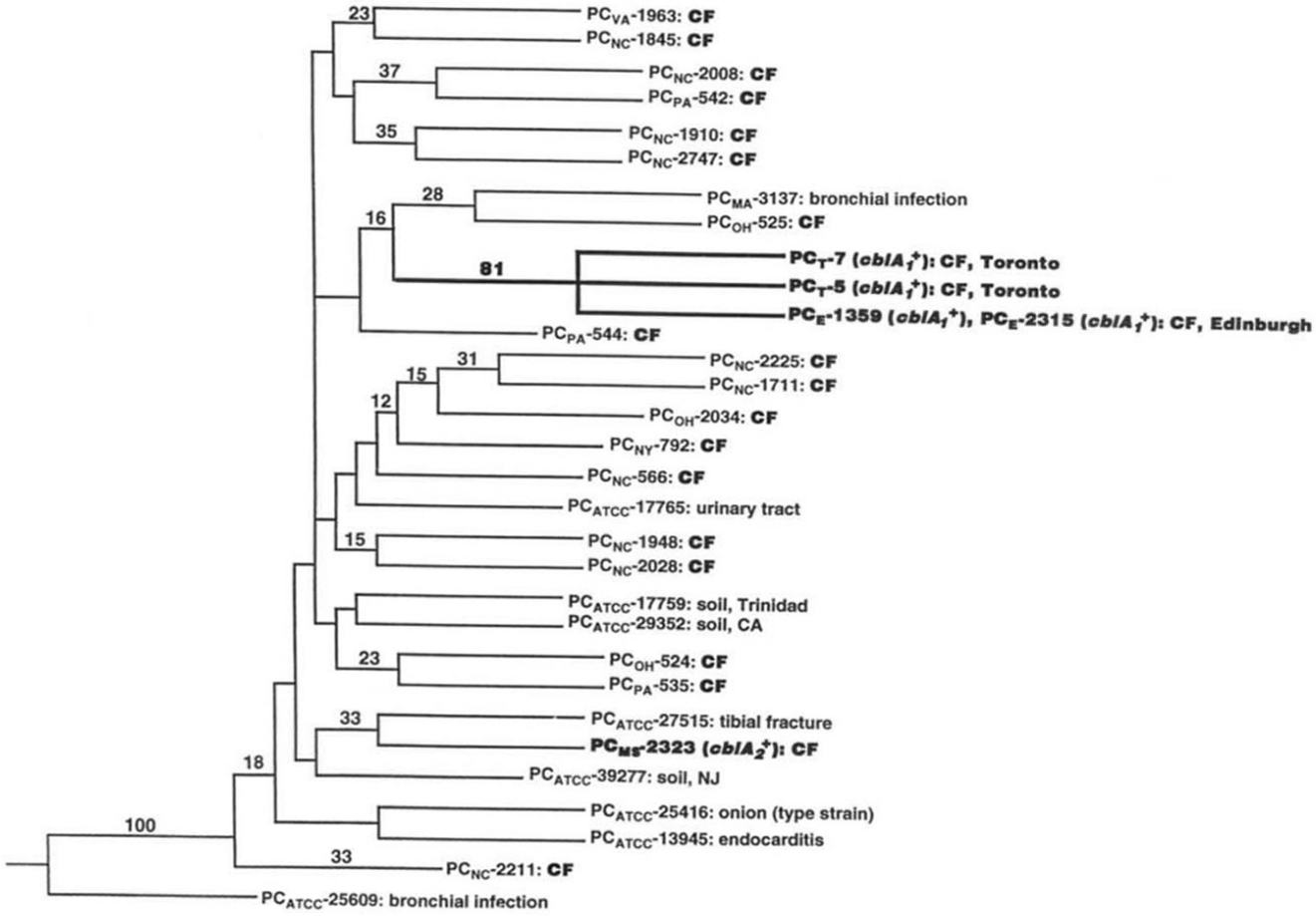

Fig. 2 *rrn*-RFLP-based phylogenetic tree of representative isolates from patients at seven CF centres in North America (Chapel Hill, North Carolina; Jackson, Mississippi; Norfolk, Virginia; Cleveland, Ohio; Philadelphia, Pennsylvania; New York, New York; Toronto, Ontario) and the United Kingdom (Edinburgh) plus environmental and clinical (non-CF) sources. All cited isolates are described in the text and in the Methods section. Indicated isolate number is followed by source (CF, environmental or clinical). *cblA$_1^+$*, *cblA$_2^+$*, isolate(s) that encode the *cblA* gene (Fig. 1*c*) and express adhesin Cbl pili (Fig. 3). *cblA$_1^+$*, identical 501-bp sequence carried by Toronto and Edinburgh CF centre isolates (Fig. 4); *cblA$_2^+$*, polymorphic 501-bp sequence carried by Jackson, Mississippi, CF centre isolate PC$_{MS}$-2323 (Fig. 4). Number above each branch indicates the percentage of time each was joined together under bootstrap analysis[16] (confidence intervals less than ten have been omitted for clarity). The lineages included in this tree are representative of the larger sample of isolates collected. Multiple CF patient serial isolates of an identical *rrn* RFLP profile have not been included as they do not affect the tree topology. However, multiple isolates from Toronto (PC$_T$-5, PC$_T$-7) and Edinburgh (PC$_E$-1359, PC$_E$-2315) CF centres are noted because further analysis by DNA sequence revealed that the *cblA* genes encoded by these four isolates are identical (Fig. 4). The remaining 13 and 11 isolates, respectively, from each of these two CF centres are members of the indicated epidemic lineage based on 100% correlation of their *rrn* RFLP profiles with those of the prototypic patterns of the Toronto/Edinburgh isolates shown in Fig. 1*b*.

fluence directly clinical management of some 70,000 CF patients in Europe and North America, we used DNA sequence analysis to test the RFLP-based conclusions that isolates from Toronto and Edinburgh were clonal. Either of two classes of bacterial genes are typically sequenced for this purpose: slowly evolving, 'housekeeping' genes such as *putP* (proline permease)[17] or, more rapidly evolving, antigen-encoding genes such as the flagellar filament gene (*fliC*) of *Salmonella typhimurium*[18]. Based on analogy to the latter, we chose the *cblA* pilin gene as it would probably be under antigenic selection, thus providing a more rigorous test for the clonality of strains. The possibility of antigenic variability being reflected in the *cblA* pilin gene sequence was suggested by our analyses of the Jackson, Mississippi, CF-associated strain PC_{MS}-2323, the sole *cblA*-positive isolate not associated with the epidemic clusters (lane 17, Fig. 1*c*). Variability in the encoded *cblA* of this isolate had been inferred by (1) ribotype and chromosomal macro-RFLP profiles indicating that PC_{MS}-2323 was only distantly related to the *cblA*-positive Toronto/Edinburgh isolates (lane 17 versus lanes 1–8 of Fig. 1*a*, *b*); (2) variation in *cblA*-encoded restriction fragment size from that observed in the Toronto/Edinburgh isolates (lane 17 versus lanes 1–8 of Fig. 1*c*) and (3) absence of agglutination by antibodies made against Cbl pili purified from *cblA*-positive Toronto CF centre isolates (data not shown).

Primers were synthesized from the *cblA* sequence of the Toronto isolate PC_T-7 (ref. 4) and used for polymerase chain reaction (PCR)-based amplification of the *cblA* gene from isolates to be characterized. Resultant PCR products were then cloned and sequenced (see Methods). Complete *cblA* sequences were thus obtained from isolates with the two slightly variant though closely related RFLP profiles typical of the 15 Toronto CF centre isolates (Fig. 1*a*, *b*, lanes 1–4), the two slightly variant though closely related RFLP profiles typical of the 13 Edinburgh CF centre isolates (Fig. 1*a*, *b*, lanes 5–8), and the significantly variant Jackson, Mississippi, CF-associated strain PC_{MS}-2323 (Fig. 1*a*, *b*, lane 17). Comparison of these five sequences indicates that the chromosomally encoded, 501-base pair (bp) *cblA* pilin subunit structural gene carried by the closely related isolates from the Toronto and Edinburgh CF centres was invariant in sequence. In contrast, the *cblA* gene encoded by the distantly related Jackson Mississippi strain PC_{MS}-2323 exhibited polymorphism at the sequence level, with changes in 60 bp of the 501-bp sequence (88% identity; see Fig. 4). The perfect conservation of the *cblA* pilin sequence among isolates from multiple patients over four years at the Toronto and Edinburgh centres is precisely what would be expected for epidemic transmission of a highly infectious clone. Likewise, the variant *cblA* encoded by the Mississippi CF centre isolate is in accord with that expected for a distantly related isolate (Fig. 2).

Discussion

P. cepacia varies in transmissibility

Consideration of these findings in the context of our previous studies on UNC CF centre isolates[9] leads us to conclude that isolates of *P. cepacia* are not equally transmissible between CF patients, rather, there exists at least one significantly divergent, highly transmissible clonal lineage plus numerous moderately heterogeneous lineages of negligible transmissibility (see Fig. 2), and that the highly transmissible lineage identified is responsible for epidemics at North American and British CF centres. This was most likely due to an as yet unidentified transatlantic transmission related to joint summer camp attendance.

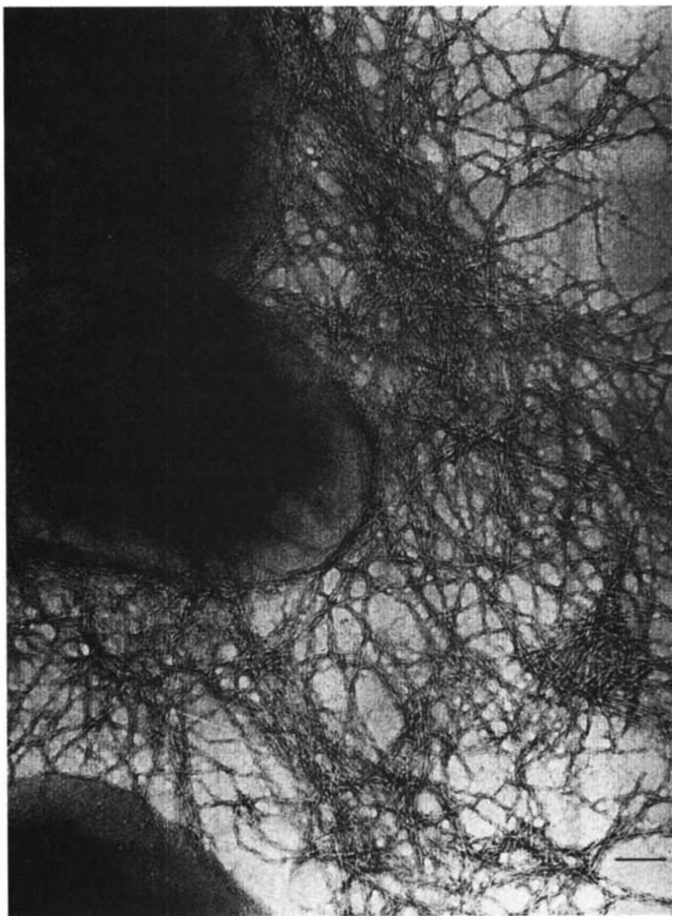

Fig. 3 Transmission electron micrograph of Toronto epidemic strain PC_T-7 expressing Cbl adhesin pili. High resolution was achieved with a JOEL 100 CX electron microscope as previously described[13]. Bar in lower right corner, 0.1 μm. Taken from Goldstein *et al*[13].

Relation of Toronto/Edinburgh clone to other epidemic strains

Based on *rrn* RFLP profiles or anecdotal evidence, additional reports suggest the occurrence of *P. cepacia* transmission at CF centres in Philadelphia[2] and Cleveland[19], respectively. We characterized strains involved with both of these putative outbreaks (see Methods section) and found that by neither ribotype or macro-chromosomal RFLPs profile did the prototypic RFLP fingerprints of the putatively epidemic strains from either centre appear similar to one another (mean $D \leq 0.3$), nor to the unique, highly transmissible lineage involved with the Toronto and Edinburgh CF patients (L.S., A.H., H. Zhou and R.G., unpublished data). Nonetheless, highly conserved RFLP profiles (mean $D \geq 0.85$) within the individual outbreaks did support a picture of epidemic transmission within each of the two centres. When these isolates were further characterized, hybridization-based survey for the presence of the *cblA* pilin gene proved negative for 35 of the involved strains (L. S., A.H., H. Zhou and R.G., unpublished data). These results suggest that there may exist *P. cepacia* lineages of high transmissibility other than the *cblA*$^+$ clone that we have identified.

Emergence of clone for efficient infection of the CF lung

This study demonstrates the integral role of molecular epidemi-

ology and evolutionary biology in identifying newly emerging, highly transmissible microbial pathogens. The degree of divergence of the *cblA*$^+$ lineage suggests specific adaptive, evolutionary changes for efficient transmission in the CF population. A number of phenotypic observations support this hypothesis, such as the novel giant Cbl pili expressed, which have been found to promote adhesion-based colonization of the CF airway[4,11,13]. New results provide further details, showing that *cblA*$^+$ isolates are significantly more adherent to human, primary culture *cftr*$^{-/-}$ airway epithelial cells than *cblA*$^-$ strains, and that the *cblA*$^+$ isolates adhered to the epithelial apical surface as well as to the cilia (J. Yankaskas, P. Gilligan and R.G., unpublished observations), suggesting the potential for interference with the mucociliary transport system. Further, unlike non-epidemic *P. cepacia* isolates, clones from the Toronto/Edinburgh epidemics proved uniquely resistant to killing by *P. aeruginosa* isolates cultured from many different patients (C. Campanelli, A.H. and R.G., unpublished observations). Given that CF patients are most often infected with *P. aeruginosa* before superinfection by *P. cepacia*, this atypical resistance may contribute to the remarkable capacity of the *cblA*$^+$ lineage to be epidemically spread among the CF population.

From molecular genetics to clinical management

Although it remains to be determined how many other divergent, highly transmissible lineages of *P. cepacia* have evolved, these results provide both the first chromosomal RFLP fingerprint (Fig. 1*a*, lanes 1–8) and DNA sequence (Fig. 4) that can be used for precise identification of one such lineage (Fig. 2). Knowledge of specific, intragenic regions of the invariant Toronto/Edinburgh *cblA* sequence allows for rapid, PCR- or oligo hybridization-based screening for this highly infectious lineage (L.S., A.H., H. Zhou and R.G., unpublished observations). The resolution of a chromosomal fingerprint unique to a particular epidemic lineage indicates that it should be possible to establish an epidemiological library of such profiles. Among the increasing population of CF patients infected with *P. cepacia*, prospective screening for the highly transmissible lineages will provide a rational basis for patient isolation and infection control policies. This is of immediate relevance to the clinical management of the CF population as such decisions impact dramatically on patients, their families and the health-care system.

Methods

Bacterial isolates. *Pseudomonas cepacia* (n = 133) isolates were obtained from the following sources: (1) 65 isolates from patients at the University of North Carolina (UNC) Cystic Fibrosis Centre (1985–93) including 17 clinic and 5 transplant patients, 4 of whom were infected transfers from other distant locations as described previously[9,13]. Those cited in the figures include isolates PC_{NC}-566, PC_{NC}-1711, PC_{NC}-1845, PC_{NC}-1910, PC_{NC}-1948, PC_{NC}-2008, PC_{NC}-2028, PC_{NC}-2211, PC_{NC}-2225 and PC_{NC}-2747, each from different local clinic patients, and four isolates from infected transfer patients (PC_{NY}-792 from New York, New York, PC_{VA}-1963 from Norfolk, Virginia, PC_{OH}-2034 from Cleveland, Ohio, and the *cblA*$^+$ isolate PC_{MS}-2323 from Jackson, Mississippi); (2) 8 ATCC clinical and environmental isolates: human endocarditis PC_{ATCC}-13945, human urinary tract PC_{ATCC}-17765, human bronchial PC_{ATCC}-25609, human tibia fracture PC_{ATCC}-27515, forest soil (Trinidad) PC_{ATCC}-17759, onion PC_{ATCC}-25416 (the ATCC *P. cepacia* type strain), soil (California) PC_{ATCC}-29352, cornfield soil (New Jersey) PC_{ATCC}-39277, all as described previously[9,13]; (3) 15 *cblA*$^+$ isolates from 15 CF centre clinic patients at the Hospital for Sick Children (1987–88), Toronto. Those cited in the figures include isolates PC_T-5, PC_T-7, PC_T-19, PC_T-25 (refs 4, 13); (4) 2 isolates, PC_E-SBC27 and PC_E-SBC29, from two CF patients at Western General Hospital (Edinburgh, Scotland) that were not associated with epidemic transmission within this CF centre[3,4,13]; (5) 4 isolates from clinic patients at Western General Hospital (1989–90), Edinburgh, all of which had been associated with epidemic transmission within this CF centre[3]. Those cited in the figures include isolates PC_E-509, PC_E-1359, PC_E-1392 and PC_E-2315; (6) 10 isolates from 10 patients at a Philadelphia, Pennsylvania, CF centre[2]. Those cited in figures include isolates PC_{PA}-535, PC_{PA}-542 and PC_{PA}-544; (7) 24 isolates from 24 patients at the Rainbow Babies and Children's Hospital, Cleveland, Ohio[19], including cited isolate PC_{OH}-524, PC_{OH}-525 and PC_{OH}-2034; and (8) 5 bronchial isolates from 5 non-CF ventilator patients at Boston City Hospital (Massachusetts) including cited isolate PC_{MA}-3137.

RFLP, amplification and sequencing analyses. PFGE, ribotype and *cblA* hybridization methods are described in detail in recent publications cited in the text[4,9,13]. Using previously described methods[9], CsCl equilibrium density gradient purified chromosomal DNA[20] was isolated from the two prototypic Toronto epidemic isolates (PC_T-7 and PC_T-5), the two prototypic Edinburgh epidemic isolates (PC_E-2315 and PC_E-1359) and the single Jackson, Mississippi, CF centre isolate

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  1 ATGCTGAAATACGTTCCGATCGCTGCTGCTGCTCTGATGTCGATGTCGGC 50
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  1 ATGCTGAAATACGTTCCGATCCGTGCCGCCGCCCTGATGTCGATGTCGGC 50

 51 TTACGCCGTCCAGAAGGACATTACCGTCACCGCCAACGTCGACACGACGC 100
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 51 TCACGCCGTGCAGAAGGACATCACCGTCACCGCCAACGTCGACACGACGC 100

101 TCGAAATGCTGTCGGCGGACGGCTCGGCACTGCCGACGACCATGCAGATG 150
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101 TCGAGATGCTGTCGGCGGACGGTTCGGCGCTGCCGACGACCATGCAGATG 150

151 CAATATCTGCCGGGTACGGGTCTTCAGGCAGCTGTAGTGAACACGAAGAT 200
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151 CAGTATCTGCCGGGCTCGGGTCTCCAGGCGGCTGAAGTGAACACGAAGAT 200

201 CTTCACGAACGACAAGGCAAAGGATCTGCAGATCCGCCTCGCGACTGCCC 250
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201 CTTCACGAACGACAAGGCGAAGGATCTGCAGATCCGCCTCGCGGCCGAGC 250

251 CGGCTTTGAAGAACCAGACGAGCCCGGGCGCGGCGGAAATTCCGCTGTCG 300
    ||||  |||||||||||||||||||||||||  ||| |||||||| ||
251 CGGCGCTGAAGAACCAGACGAGCCCGGGCGCCAAGGAGATTCCGCTGGCG 300

301 GTCAAGCTTGGCGAAACCGAGCTGACCACCACGGCCGCGACGCTGAAGAC 350
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301 GTCAAGCTGGGCACCACCGCGCTGAGCACCACGGCGACGACGCTGAAGGC 350

351 CGCAGAGCTCTTCCCCGGCGAACTGGCACAAGGTTCGAACGTGCTGGCGC 400
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351 GTCGGAGATCTTTACCGGTGAACTGGCGCAGGGCTCGAACGTGCTGCCGC 400

401 TGTCGATCGGTCAGAAGAAGGTCGAAGCCGTCACGGCGTCCGGCAGCTAC 450
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401 TGTCGATCGAACAGAAGACGGCCGGAGCCATCACGGCTGCCGGCAGCTAT 450

451 CAGGGCCTCGTCAGCGTGATCGTCACGCAGAGCGCGGCTTCGGGTAGCTA 500
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451 CAAGGCCTCGTCAGCGTGATCGTCACGCAGAGCGCGGCATCGGGTAGCTA 500

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Fig. 4 Identical 501-bp sequence (topmost line) of the *cblA* structural gene encoded by two prototypic Toronto epidemic isolates (PC_T-7 and PC_T-5) and two prototypic Edinburgh isolates (PC_E-2315 and PC_E-1359) compared to the variant *cblA* sequence carried by the single Jackson, Mississippi, CF centre isolate PC_{MS}-2323 (lower line). See Methods section.

PC_{MS}-2323. From each of these *cblA* probe-positive chromosomes, the *cblA* gene was PCR amplified[21] using a DNA thermocycler (Perkin-Elmer) with a GeneAmp PCR Core Reagents Kit (*ibid.*). Based on the previously determined sequence of the *cblA* gene encoded by isolate PC_T-7 (ref. 4), sense and antisense primers used for these reactions were 5′–CCAAAGGACTAACCCA–3′ and 5′–ACGCGATGTCCATCACA–3′, respectively. PCR reactions were as follows: cycle one, 2 min at 94 °C, 2 min at 37 °C, 1 min at 72 °C. The remaining 29 cycles were: 1 min at 94 °C, 1 min at 45 °C, 1 min at 72 °C, followed by 7-min extension at 72 °C. PCR products were separated by electrophoresis through 0.8% agarose and for each a single band was observed with ethidium bromide staining. Bands were electroeluted into DEAE membrane (Schleicher & Schuell) and cloned with a TA Cloning Kit (Invitrogen). DNA sequences were determined by the Sanger dideoxy method[22] with the same primers used for PCR amplification (above). Five PCR-amplified *cblA* gene clones of PC_{MS}-2323 were generated, three of which were sequenced for confirmatory purpose, with no variation resolved.

Statistics. Standard criteria were used for comparing PFGE patterns[23]. According to established criteria for *P. cepacia*[24], strains were assigned to the same ribotype when comparison of sizes of hybridizing fragments revealed three or fewer bands differing between the two patterns under comparison. Quantitative pairwise comparison of both types of RFLP patterns was accomplished using the Dice coefficient of similarity calculated as $D = 2n_{xy} / (n_1 + n_2)$, where n_1 is the total number of DNA fragments from strain *X*, n_2 is the total number from strain *Y*, and n_{xy} the number of fragments identical in the two strains[14,25]. The coefficient of similarity for two PFGE RFLPs $D \geq 0.90$ represents closely related strains, while unrelated strains have $D \leq 0.60$. Intervening values, remarkably, are rare[9]. For *rrn* RFLPs, given that *P. cepacia* strains typically display 7–10 distinct hybridizing bands, the shared ribotype (above) would correspond to D = 0.79 to 0.85. Comparisons between mean values were performed by Student's *t*-test using a Systat program (Systat Inc.).

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