Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 311 (2003) 665–671
BBRC
www.elsevier.com/locate/ybbrc

Unusually common cystic fibrosis mutation in Portugal encodes a misprocessed protein

Filipa Mendes,[a] Mónica Roxo Rosa,[a,c] Anca Dragomir,[b] Carlos M. Farinha,[a,c] Godfried M. Roomans,[b] Margarida D. Amaral,[a,c] and Deborah Penque[a,*]

[a] *Centro de Genética Humana, Instituto Nacional de Saúde Dr Ricardo Jorge, Lisboa 1649-016, Portugal*
[b] *Department of Medical Cell Biology, University of Uppsala, Uppsala, Sweden*
[c] *Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Portugal*

Received 8 October 2003

Abstract

A561E, a novel cystic fibrosis (CF) associated mutation in the first nucleotide binding domain of CFTR, is the second most common CF mutation in Portugal. Properties of the A561E-CFTR protein were studied by immunoblotting, pulse-chase, immunocytochemistry, and MQAE halide-efflux assay in stably transfected BHK cells. Altogether, results presented here suggest that A561E causes protein mislocalization in the endoplasmic reticulum where the mutant protein must be trapped by the quality control mechanism. We conclude that A561E originates a protein trafficking defect, thus belonging to class II of CFTR mutations. As it is the case for F508del-CFTR (the most common CF mutant), low temperature treatment partially rescues a functional A561E-CFTR channel, suggesting that substitution of glutamic acid for alanine at position 561 does not completely abolish CFTR function. Pharmacological strategies previously reported for treatment of CF patients with the F508del mutation could thus be also effective in CF patients bearing the A561E mutation.

Keywords: Cystic fibrosis; CFTR; A561E mutation; Classes of CFTR mutations

Cystic fibrosis (CF) is a common autosomal recessive inherited disorder in the Caucasian population [1,2]. The disease is caused by mutations in the gene coding for the CF transmembrane conductance regulator (CFTR), a cAMP-regulated chloride channel that is functional in the apical surface of epithelial cells [3,4]. About one thousand different disease-causing mutations that affect different aspects of CFTR function have been reported in the CFTR gene (http://www.genet.sickkids.on.ca/cftr). The predominant mutation in the CF population, found in more than 70% of CF patients in Northern Europe and North America, is the deletion of phenylalanine at position 508 (F508del) in the first nucleotide binding domain (NBD1) [5]. However, in Portugal, the F508del mutation accounts for only 52% of the CF genes analysed, the remaining mutations are mostly rare or even unique to the Portuguese CF population [6]. One of them is the novel CF missense mutation A561E, in which alanine is replaced by glutamic acid at position 561 of the CFTR polypeptide (http://www.genet.sickkids.on.ca/cftr). The A561E mutation accounts for 3% of Portuguese CF genes, being the second most frequent CF mutation in Portugal. Four CF patients have so far been identified as homozygous for this mutation (Pacheco et al., personal communication).

CFTR mutations have been divided into four classes [7]. Class I mutations (stop and splicing mutations) prevent or diminish synthesis of the CFTR protein. Class II mutations are defective in intracellular trafficking as the translated immature forms of these CFTR mutants are mostly retained in the endoplasmic reticulum (ER) failing to mature into fully glycosylated forms at the cell membrane. F508del is the prototype example of the class II mutation. Class III mutations allow CFTR to be placed at the cell surface but impair its regulation or cAMP-mediated activation of Cl^- transport. In Class IV mutations, the CFTR is normally

* Corresponding author. Fax: +351-21-752-64-10.
E-mail address: deborah.penque@insa.min-saude.pt (D. Penque).

0006-291X/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2003.10.048

localized but has reduced Cl^- transport in response to cAMP stimulation.

Recognition of distinct classes of CFTR mutations has to some extent improved the ability to predict the course of CF in individual patients and has also stimulated the design of therapeutic strategies directed at correcting the particular impairment of a specific mutation [8].

The aim of the present work was to classify the A561E mutation into one of the functional defect classes of CFTR mutations. Therefore, we have stably overexpressed A561E CFTR in a heterologous expression system, baby hamster kidney (BHK) cell lines, and analysed them by immunoblotting, pulse-chase, halide sensitive fluorescent dye assay, and immunocytochemistry in order to characterize the molecular mechanism of the A561E mutation. The classification of A561E mutation into one of the classes of CFTR mutations will allow a better understanding of the relationship between the functional alterations and disease phenotype and pave the way for designing appropriate pharmacological interventions in CF patients bearing this particular mutation.

Materials and methods

Site-directed mutagenesis and expression construct. CFTR cDNA wild-type (pBQ 4.7) was generously provided by J. Rommens and L.C.-Tsui (Toronto, Canada). A561E mutation was created in pBQ 4.7 using Muta-gene phagemid in vitro mutagenesis Kit (BioRad Laboratories, Hercules, CA, USA) according to manufacturer's recommendations. The oligonucleotide 5′-CTTTAGCAAGAGAAG TATACAAAGATGC-3′ was used to produce A561E mutation and the mutants were subsequently confirmed by DNA sequence analysis. Full-length fragment cut off from A561E *CFTR* cDNA/pBQ4.7 was subcloned into the eukaryotic expression vector pNUT (kindly provided by J. Riordan, Scottsdale, USA).

Production of CFTR expressing BHK. Baby hamster kidney (BHK) cells were stably transfected with A561E-CFTR pNUT recombinant vector by using DOTAP (Boehringer Mannheim GmbH, Mannheim, Germany) following the manufacturer's recommendations. After 48 h, 500 μM methotrexate was added to the medium. The resistant clones were isolated about 4 weeks later and the CFTR expression was screened by Western blotting as described below.

BHK cells stably expressing wild-type (wt)-, F508del-CFTR (both kindly provided by G. Lukacs, Toronto, Canada) or A561E-CFTR were cultivated as described [9,10].

Western blotting. Cells were lysed with Laemmli sample buffer (1.5% SDS, 10% glycerol, 0.5 mM dithiothreitol, 31.25 mM Tris, pH 6.8, and 0.001% bromophenol blue) and total protein extracts were analysed after separation by SDS–PAGE on 7% or 6% polyacrylamide mini-gels followed by transfer onto nitrocellulose filters. The filters were probed with M3A7 anti-CFTR monoclonal antibody (mAb) (Chemicon, International, Temecula, CA) and developed using the ECL detection system (Amersham Biosciences, Uppsala, Sweden).

Pulse-chase and CFTR immunoprecipitation. Metabolic labelling and immunoprecipitation were carried out essentially as described [11]. After incubation in methionine-free α-minimal essential medium (MEM) for 30 min, cells were pulse-labelled in the same medium containing 100 μCi/ml [^{35}S]methionine (>1000 Ci/mmol; ICN Biomedicals, Irvine, CA, USA) for 30 min at 37 °C. For chasing, the labelling medium was replaced with 5% serum and 1 mM methionine at indicated times. Cells were then lysed in 1 ml RIPA buffer (150 mM NaCl, 50 mM Tris, 1% deoxycholic acid; 1% Triton X-100, and 0.1% SDS, pH 8) supplemented with protease inhibitors (10 μg/ml leupeptin and pepstatin and 0.5 mM Pefabloc, Boehringer Mannheim). Immunoprecipitates obtained with M3A7 anti-CFTR mAb were analysed by SDS–PAGE and fluorography. Fluorograms were analysed by ImageMaster software (Amersham Bioscience). Comparisons between slopes of lines representing degradation rates (regression lines) were made using a Student's t test as described before [11].

Immunocytochemistry. Cells (2×10^4) were grown on 8-well chamber slides (Nalge Nunc, Roskilde, Denmark) at 37 or 26 °C for 48 h, rinsed twice with cold phosphate buffered saline (PBS) and fixed in 4% formaldehyde, 3.7% sucrose in PBS for 30 min at 4 °C. After two washes with PBS, cells on slides were permeabilized with 0.2% Triton X-100 in PBS for 20 min and washed three times in PBS for 5 min each, and antigen was blocked with 1% bovine serum albumin (BSA)/PBS for 45 min prior to incubation overnight at 4 °C with M3A7 anti-CFTR mAb. Cells were then washed three times with PBS, for 10 min each, and incubated with the fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Jackson ImmunoResearch Laboratories, Baltimore, MD, USA) diluted at 1:100 for 45 min at RT. The slides were mounted with Vectashield (Vector Laboratories, Burlingame, CA, USA) containing DAPI (4,6-diamino-2-phenylindole, from Sigma, St. Louis, MO, USA) for nuclei staining and covered with a glass coverslip. Immunofluorescence staining was observed and recorded on an Axioskop fluorescence microscope (Zeiss, Jena, Germany) with the Power Gene 810/Probe & CGH software system (PSI, Chester, UK).

MQAE fluorescence assay. For the Cl^- efflux experiments, cells were grown to confluence on glass coverslips. As cells do not multiply at lower temperature (26 °C) they were first grown to confluence under regular conditions (37 °C) and then transferred to lower temperature.

Cells were loaded with 10 μM MQAE (*N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide, from Molecular Probes, Eugene, OR, USA) in culture medium, for 2–4 h at 37 or 26 °C, respectively. The coverslips were placed on the bottom of a perfusion chamber on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan). Cells were then perfused continuously with buffer pre-warmed at the desired temperature.

The experiments were performed by sequential exposure of cells to a Cl^--rich buffer containing 140 mM NaCl, 5 mM KCl, 5 mM Hepes, 1 mM $MgCl_2$, and 5 mM glucose, pH 7.4, followed by exposure to a Cl^--free buffer of similar composition, but with NO_3^- as the substituting anion (basal efflux). Cells were allowed to recover by perfusing them with the Cl^--rich buffer and cAMP-stimulated Cl^- efflux was determined by exposure to Cl^--free buffer containing 5 μM forskolin and 100 μM IBMX (both from Sigma).

For the intracellular calibration, a K^+-rich buffer containing 120 mM K^+, 5 mM Hepes, 5 mM glucose, 1 mM Mg^{2+}, and various concentrations of Cl^- and NO_3^- was used at pH 7.2, in the presence of the ionophores 10 μM tributyltin (Aldrich-Chemie, Steinhem, Germany) and 10 μM nigericin (Molecular Probes). The autofluorescence of the cells was recorded in a quenching solution containing 150 mM KSCN and 10 mM Hepes, pH 7.2.

A Quanticell2000 image-processing system (VisiTech International, Sunderland, UK) provided the excitation light at 355 nm wavelength (20 nm bandwidth) for 16 ms at an interval of 3–7 s. The emission light was measured at 460 nm (30 nm bandwidth) using an analogue CCD camera.

The fluorescence was transformed into Cl^- concentration using the results of the intracellular calibration as described elsewhere [12]. For each experiment, all cells in the optic field were analysed (20–40 cells) and their average response was counted as one experimental data point. The software GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA) was used to determine the maximal value of the Cl^- efflux rate. Four to 10 experiments were performed for each condition tested and results followed a normal distribution. For statistical

analysis, one-way ANOVA for repeated measurements was used, followed by Bonferroni's post test.

Results

A561E-CFTR is a misprocessed protein

The A561E mutation was subcloned into the higher eukaryotic expression vector pNUT and the recombinant vector was stably expressed in BHK cell lines. Fifteen to 20 clones were evaluated by Western blotting using the M3A7 CFTR antibody and one of the positive clones expressing high amounts of A51E-CFTR was chosen for the subsequent experiments in order to study the biosynthetic process of this mutant.

Fig. 1A shows the Western blotting of a total protein extract from one of these BHK cell clones stably expressing A561E analysed in parallel with extracts of BHK cells expressing wt- or F508del-CFTR as controls. Like F508del-CFTR (Fig. 1A, lane 3), A561E-CFTR is only detected as an immature, ER core-glycosylated form of CFTR (band B), of 150 kDa in this heterologous expression cell system (Fig. 1A, lane 4). No mature complex-glycosylated forms (band C) of 170–180 kDa were detected for A561E-CFTR. Mature CFTR that trafficked to the Golgi, where complex oligosaccharide processing takes place, was only detected in protein extracts of BHK cells expressing wt-CFTR (Fig. 1A, lane 2).

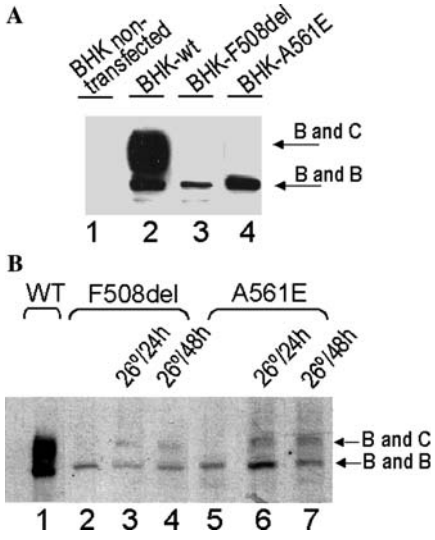

Fig. 1. Western-blotting analysis of BHK cells stably expressing wt-, F508del- or A561E-CFTR. (A) Effect of A561 mutation in the maturation of CFTR. Cell lysates were prepared (see Materials and methods) from BHK cells non-transfected (50 μg, lane 1), or stably transfected with wt- (30 μg, lane 2), F508del- (50 μg, lane 2) or A561E-CFTR cDNA cloned into pNUT vector (50 μg, lane 4), and resolved on a 6% SDS–polyacrylamide gel before electrophoretic transfer to nitrocellulose for immunodetection of CFTR, using M3A7 anti-CFTR antibody (1:1000). The complex-glycosylated forms of CFTR (band C, see arrow) are absent in cells expressing A561E or F508del-CFTR. (B) Effect of low temperature on the trafficking defect of A561E- and F508del-CFTR. BHK cells stably expressing F508del- or A561E-CFTR were incubated at 26 °C for 24 h (100 μg/per lane, lanes 3 and 6, respectively) or for 48 h (100 μg/lane, lanes 4 and 7, respectively) prior to evaluation by Western-blotting (as described in (A)), for the presence of mature or immature A561E- or F508del-CFTR. To facilitate immunodetection of the rescued mutant, cells were incubated for 1 h with cycloheximide (100 μg/ml) prior to analysis. As controls, cell lysates from BHK cells expressing wt- (30 μg, lane 1), F508del- (50 μg, lane 2) or A561E-CFTR (50 μg, lane 5) grown at 37 °C were also analysed in parallel. The abundance of complex-glycosylated forms of wt-, F508del-, and A561E-CFTR persisting in the cell (indicated by arrows) was calculated from densitometry of immunoblots.

Immunolocalization studies revealed that in contrast to the wt-CFTR, which is essentially detected in the cell membrane (Fig. 2A), A561E-CFTR shows the same prominent ER localization (Fig. 2C) as F508del-CFTR (Fig. 2B). To assess whether a small amount of A561E-CFTR, below the biochemical and immunocytochemical detection limits, traverses the Golgi to the plasma membrane, we employed the more sensitive single-cell membrane halide permeability assay using the Cl^- indicator MQAE. Fig. 3A (upper panel) shows a rapid change in MQAE fluorescence on stimulation with cAMP agonists in BHK cells expressing wt-CFTR indicating the presence of functional CFTR at the plasma membrane. By contrast, no measurable cAMP-stimulated Cl^- channel activity was detected in cells expressing A561E- or F508del-CFTR (Fig. 3A, middle and lower panels, respectively).

Taken together, these data demonstrate that A561E-CFTR is misprocessed and retained intracellularly, thus failing both to localize correctly and to function at the plasma membrane. Like F508del, the A561E should thus be included into class II of defective processing CFTR mutations.

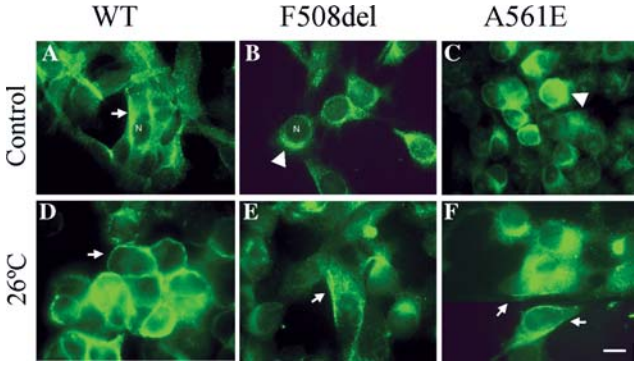

Fig. 2. Immunolocalization of CFTR in BHK cells expressing wt-, F508del-, or A561E-CFTR. Cells were grown in 8-well chamber slides and incubated at 37 °C (A–C) or at 26 °C for 24 h (D–F) prior to analysis by immunocytochemistry (see Materials and methods) using M3A7 anti-CFTR antibody (1:50). At normal temperature of cell culture (37 °C), A561E-CFTR (C) is detected strictly in the area around the nuclei of the cells like F508del-CFTR (B); while wt-CFTR (A,D) is predominantly located at the plasma membrane. At low temperature (26 °C), some A561E- and F508del-CFTR are rescued to the cell surface (see arrows head in (E) and (F)).

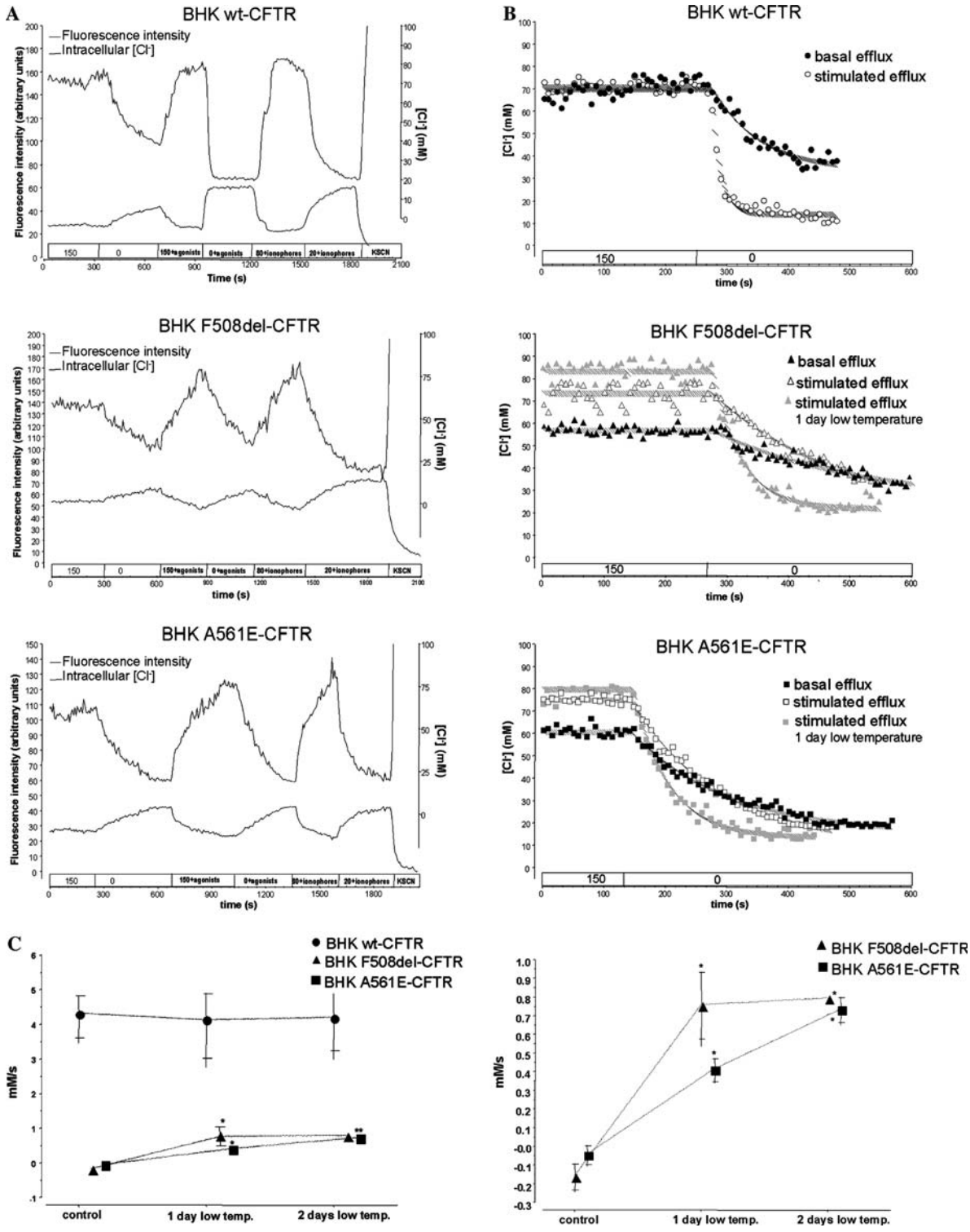

Fig. 3. Chloride efflux studied by MQAE fluorescence. (A) Characteristic records of MQAE fluorescence (thin lines) and corresponding intracellular Cl^- concentration (thick lines) in BHK cells stably expressing wt-, F508del- or A561E-CFTR. The rectangle above the time axis indicates the concentration of Cl^- (in mM) in the extracellular solution bathing the cell. It also indicates the presence of agonists (5 μM forskolin + 100 μM IBMX) and ionophores (10 μM tributyltin + 10 μM nigericin). (B) Effect of low temperature on Cl^- concentration and efflux in BHK cells stably expressing wt-, F508del- or A561E-CFTR. The Cl^- efflux was induced by changing the concentration of Cl^- in the extracellular solution bathing the cells as indicated by the rectangles above the time axis, in the absence or presence of cAMP agonists (basal, respectively, stimulated efflux). Experimental data are displayed by indicated symbols while the continuous lines were obtained by computer fitting to an exponential function. (C) Effect of low temperature incubation on the cAMP-stimulated Cl^- efflux rates. The basal efflux values were subtracted from the stimulated efflux values for each individual experiment (matched data) and results are illustrated as means ± standard error of the mean. Significant difference from control (see Materials and methods) is indicated by * ($p < 0.05$) and ** ($p < 0.01$).

The turnover rates of A561E-CFTR are similar to those of F508del-CFTR

To study whether A561E-CFTR and F508del-CFTR have the same biogenesis and kinetics degradation, we analysed these two mutants and the wt-protein by pulse-chase technique. After pulse labelling the transfected cells for 30 min with [^{35}S]methionine, CFTR was immunoprecipitated and analysed by SDS–PAGE (Fig. 4A). As reported earlier [11,13,14], newly synthesized wt- and F508del-CFTR (0 min chase) migrate as multiple bands of 130–150 kDa (also known as band B). The newly synthesized immature form of A561E-CFTR migrates with the same molecular mass of wt- and F508del-CFTR (Fig. 4A).

Following the chase (0.5, 1, 2, and 3 h), the core-glycosylated form of wt-CFTR disappears gradually, with a half-life ($t_{1/2}$) of 30–45 min. A fraction of the labelled protein (~50%) is converted to a species of higher molecular mass (170–180 kDa, also known as band C) after 3 h of chase.

The pulse-labelled core-glycosylated F508del-CFTR is not processed to the complex-glycosylated species in these cells and almost disappears after 2 h. Here, we observed the same phenomenon for A561E-CFTR. Multiple pulse-chase experiments ($n = 3$) indicate that the $t_{1/2}$ of immature core-glycosylated A561E-CFTR was very similar to that of F508del-CFTR, around 40 min, which is slightly lower than that of the wt CFTR in BHK cells as described by Luckas et al. [11,14] (Fig. 4C).

As the immature A561E-CFTR is not converted into band C, its turnover corresponds solely to protein degradation. Therefore, we suggest that the A561E mutation does not significantly alter the susceptibility to degradation of the newly synthesized protein.

Low temperature partially restores A561E-CFTR trafficking defect

Reduced temperature is thought to partially revert the folding defect of F508del-CFTR and thus promote the traffic of functional channels to the cell surface [15–18].

To examine whether the processing defect of A561E-CFTR can be overcome by low temperature treatment, similar to F508del-CFTR, BHK cells overexpressing this mutant were incubated at 26 °C for 24 or 48 h and analysed by Western blotting (Fig. 1B). To facilitate immunodetection of the rescued mutant protein, cells were incubated for 1 h with a protein synthesis inhibitor, cycloheximide (100 μg/ml), before analysis [18]. Fig. 1B shows that mature forms of A561E and F508del-CFTR (band C) were readily detected in cells incubated at 26 °C for 24 and 48 h (lanes 6/7 and 3/4, respectively). Densitometric analysis revealed that the amount of rescued band C of either A561E or F508del does not increase upon longer incubation (48 h) at 26 °C. This result suggests that incubation at low temperature for longer periods does not cause a significant cumulative rescue effect on those CFTR mutants.

At low temperature, some A561E-CFTR and F508del-CFTR were also detectable by immunocyto-

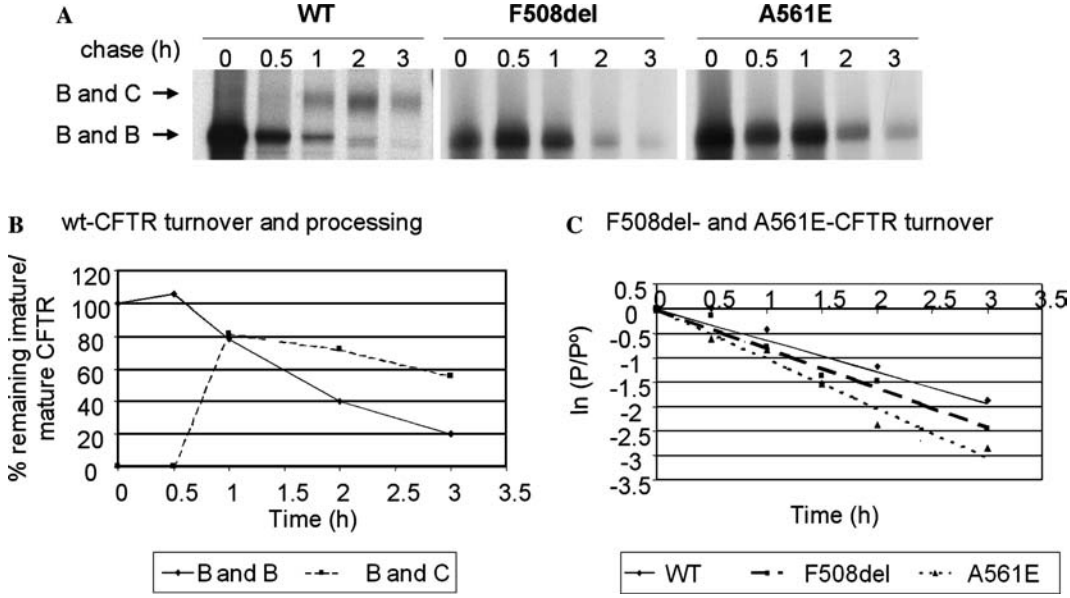

Fig. 4. Turnover rates of Wt, F508del- and A561E-CFTR in BHK cells. (A) Pulse-chase experiments followed by immunoprecipitation of wt-, F508del-, and A561E-CFTR. F508del- and A561E-CFTR proteins are synthesized as 140 kDa precursors that are very rapidly ($t_{1/2} < 30$ min) degraded with no apparent conversion to mature forms (fully glycosylated) in contrast to wt-CFTR. (B) Quantification of fluorographic data of F508del-CFTR and A561E-CFTR experiments ($n = 3$/each). P is the amount of protein at time t and P_0 is the amount of protein at the start of the experiment (chase $t = 0$). No significant differences are found between the turnover kinetics of immature forms of both F508del- and A561E-CFTR.

chemistry at the plasma membrane of the cells (Figs. 2E and F).

Additionally, the MQAE fluorescence assay also demonstrates that cells expressing A561E- or F508del-CFTR generate cAMP-stimulated Cl^- efflux after incubation at low temperature for 24 or 48 h (Fig. 3B), albeit to a lesser extent than that observed for wt-CFTR (Fig. 3C). The low temperature treatment slightly decreases cAMP-stimulated Cl^- efflux in cells expressing wt-CFTR. However, the difference in the amplitude of response to cAMP agonists between the cells expressing wt-CFTR and cells expressing CFTR mutants remains significant, even following growth at low temperature suggesting that low thermal treatment only partially corrects the trafficking defect of both A561E- and F508del-CFTR.

Taken together all data strongly indicate that A561E-CFTR is a temperature-sensitive traffic mutant. Functional cAMP-stimulated A561E-CFTR Cl^- channel activity in the plasma membrane can be promoted by reduced temperature treatment. The substitution of glutamic acid for alanine at position 561 of CFTR does not completely abolish CFTR function once the trafficking defect is corrected.

Discussion

New pharmacological therapies targeted towards specific, relatively common CFTR mutations are being developed for the treatment of CF [8,19,20]. The majority of CF patients are compound heterozygous for F508del, a class II mutation described to cause major protein misfolding and premature degradation in the ER [15]. However, in addition to F508del, about one thousand mutations can impair the cAMP-dependent CFTR Cl^- activity by a variety of mechanisms. Classifying these mutations by the nature of the resulting defect stimulates the design of specific therapeutic strategies that will certainly be beneficial to a broad range of CF patients.

The novel missense mutation A561E, located in exon 12, is part of a cluster of missense mutations that affect the highly conserved amino acid residues, between the signature (C) and Walker B motifs within the NBD1 of CFTR (Fig. 5). In Portugal, A561E is the second most frequent CF mutation, accounting for 3% of CF alleles. To date, 14 patients carrying A561E were identified in Portugal; nine are compound heterozygotes with F508del, one with G542X and four are homozygous for A561E mutation (Pacheco et al., personal communication and manuscript in preparation). The clinical features of these patients seem indistinguishable from those of F508del homozygotes, except for the fact that they show less severe pulmonary disease (C. Barreto and H. Rocha personal communications). Functional assessment of native colonic epithelia of patients, including

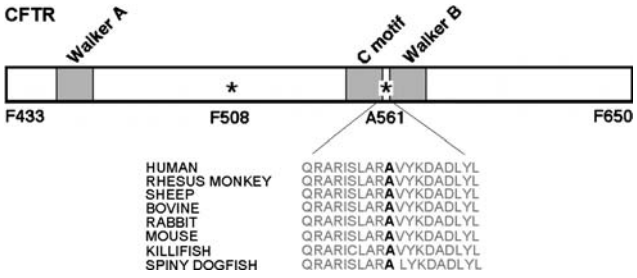

Fig. 5. F508del- and A561E-CFTR mutations in NBD1. Positions of F508 and A561 amino acid residues within CFTR NBD1 domain are indicated by asterisks. The alanine residue at position 561 of CFTR protein is highly conserved among different organisms (adapted from *CFTR protein sequence for 15 species*, http://central.igc.gulbenkian.pt/cftr//downloads/downloads.html).

one homozygote for A561E and another compound heterozygote for A561E and F508del, showed that CFTR-mediated Cl^- secretion is absent in the colon of these patients (Hirtz et al. 2003, submitted for publication) [21].

In order to explore the molecular mechanism that leads to defective Cl^- transport in A561E patients we have studied the processing, localization, and function of A561E-CFTR stably overexpressed in BHK cell lines. The results presented here clearly demonstrate that A561E has a trafficking defect when heterologously expressed in these cells. Like F508del, A561E-CFTR is not processed correctly and, as a consequence, is not delivered to the plasma membrane of these cells. Therefore, A561E mutation belongs to the class II of CFTR mutations.

In BHK cells, A561E-CFTR must not acquire its fully folded native conformation. Indeed, placing the A561 residue in the homology model of CFTR NBD1, Dorwart et al. [22] recently suggested that this residue is deeply buried in this domain and that the A561E mutation probably disrupts its efficient folding. The core-glycosylated immature form of A561E-CFTR must thus be retained in the ER by its quality control from where it is degraded.

The properties of A561E-CFTR revert towards those of wt-CFTR as the incubation temperature is reduced to 26 °C. When the processing defect is (partially) corrected, functional cAMP-regulated Cl^- channels appear in the plasma membrane, indicating that A561E mutation does not completely abolish CFTR function.

Based on the results reported here, we hypothesize that A561E-CFTR can be also re-directed to the normal protein trafficking pathway by manipulation of chaperone protein/CFTR interactions, with chemical chaperones or other drugs that affect gene regulation such as genistein and xanthine derivatives [20]. Thus, the pharmacological therapies that have been tried for CF patients bearing F508del could be also useful to those with the A561E mutation.

Acknowledgments

We thank A. Simas and I. Carvalho Oliveira for expert technical assistance, P. Pacheco (INSA, Lisboa, Portugal), C. Barreto (Hospital Sta Maria, Lisboa, Portugal), and H. Rocha (Hospital Maria Pia, Porto, Portugal) for sharing unpublished data, J.R. Riordan (Mayo Clinic, Arizona, USA) for pNUT vector, Rommens and L.C.-Tsui (Sick Children Hospital, Toronto, Canada) for *CFTR* cDNA wild-type (pBQ 4.7), G. Lukacs (University Toronto, Canada) for wt- and F508del-CFTR BHK stable cell lines, and H. Davidson (Univ. Edinburgh, UK) and J. Lavinha (INSA, Lisboa, Portugal) for manuscript discussion and English review. Work was partially supported by PRAXIS XXI P/SAU/55/96 and POCTI/MGI/47382/2000 of FCT and FEDER (Portugal) research grants, and by the Swedish Heart Lung Foundation and the Swedish Science Research Council (G.M.R.). F.M. and M.R.R. are recipients of PRAXIS XXI BD/21440/99 and BD/19869/99 doctoral fellowship, respectively. F.M. was the recipient of a travel grant from the European CF Network (EU-QLK3-1999-00241).

References

[1] J.R. Riordan, Annu. Rev. Physiol. 55 (1993) 609–630.
[2] J. Zielenski, L.C. Tsui, Annu. Rev. Genet. 29 (1995) 777–807.
[3] M.P. Anderson, D.P. Rich, R.J. Gregory, A.E. Smith, M.J. Welsh, Science 251 (1991) 679–682.
[4] C.E. Bear, C.H. Li, N. Kartner, R.J. Bridges, T.J. Jensen, M. Ramjeesingh, J.R. Riordan, Cell 68 (1992) 809–818.
[5] T.J. Sferra, F.S. Collins, Annu. Rev. Med. 44 (1993) 133–144.
[6] A. Duarte, C. Barreto, L. Marques-Pinto, M.C. Tavares, J. Amil, M. Pinto, M.L. Chieira, S. Castedo, J. Lavinha, Hum. Genet. 85 (1990) 404–405.
[7] M.J. Welsh, A.E. Smith, Cell 73 (1993) 1251–1254.
[8] M. Lim, P.L. Zeitlin, Paediatr. Respir. Rev. 2 (2001) 159–164.
[9] X.B. Chang, J.A. Tabcharani, Y.X. Hou, T.J. Jensen, N. Kartner, N. Alon, J.W. Hanrahan, J.R. Riordan, J. Biol. Chem. 268 (1993) 11304–11311.
[10] M. Haardt, M. Benharouga, D. Lechardeur, N. Kartner, G.L. Lukacs, J. Biol. Chem. 274 (1999) 21873–21877.
[11] C.M. Farinha, P. Nogueira, F. Mendes, D. Penque, M.D. Amaral, Biochem. J. 366 (2002) 797–806.
[12] P.Y. Chen, N.P. Illsley, A.S. Verkman, Am. J. Physiol. 254 (1988) F114–F120.
[13] S. Pind, J.R. Riordan, D.B. Williams, J. Biol. Chem. 269 (1994) 12784–12788.
[14] G.L. Lukacs, A. Mohamed, N. Kartner, X.B. Chang, J.R. Riordan, S. Grinstein, EMBO. J. 13 (1994) 6076–6086.
[15] G.M. Denning, M.P. Anderson, J.F. Amara, J. Marshall, A.E. Smith, M.J. Welsh, Nature 358 (1992) 761–764.
[16] M.E. Egan, E.M. Schwiebert, W.B. Guggino, Am. J. Physiol. 268 (1995) C243–C251.
[17] P.J. French, J.H. van Doorninck, R.H. Peters, E. Verbeek, N.A. Ameen, C.R. Marino, H.R. de Jonge, J. Bijman, B.J. Scholte, J. Clin. Invest. 98 (1996) 1304–1312.
[18] M. Sharma, M. Benharouga, W. Hu, G.L. Lukacs, J. Biol. Chem. 276 (2001) 8942–8950.
[19] H.C. Rodgers, A.J. Knox, Eur. Respir. J. 17 (2001) 1314–1321.
[20] G.M. Roomans, Expert. Opin. Investig. Drugs 10 (2001) 1–19.
[21] S. Hirtz, T. Gonska, H.H. Seydewitz, J. Thomas, P. Greiner, J. Kuehr, M. Brandis, I. Eichler, H. Rocha, A.-I. Lopes, C. Barreto, M.D. Amaral, K. Kunzelmann, M. Mall, (2003) submitted.
[22] M. Dorwart, P. Thibodeau, P. Thomas, CF Eur. Network Newslett. 3 (2002) 1–6.