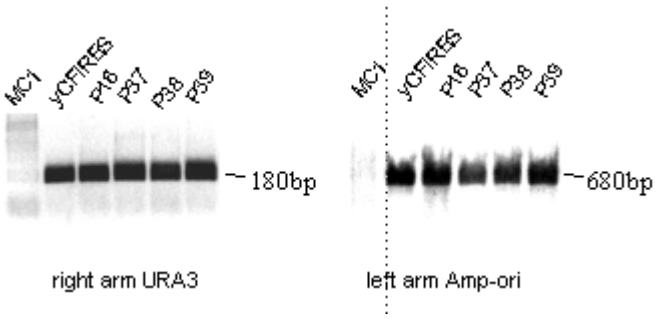
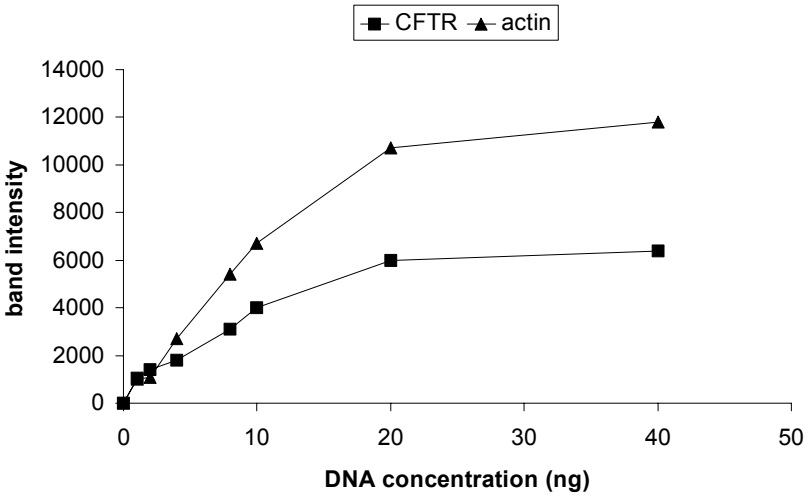
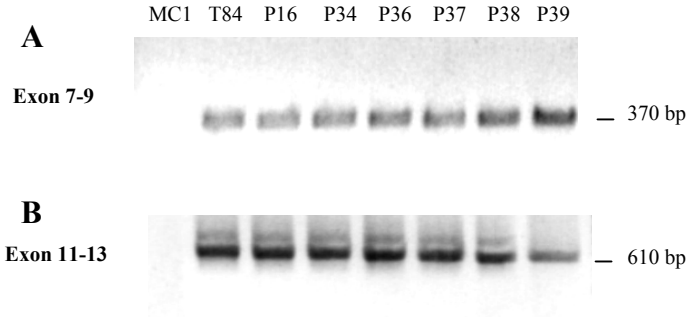
SUPPLEMENTARY MATERIAL



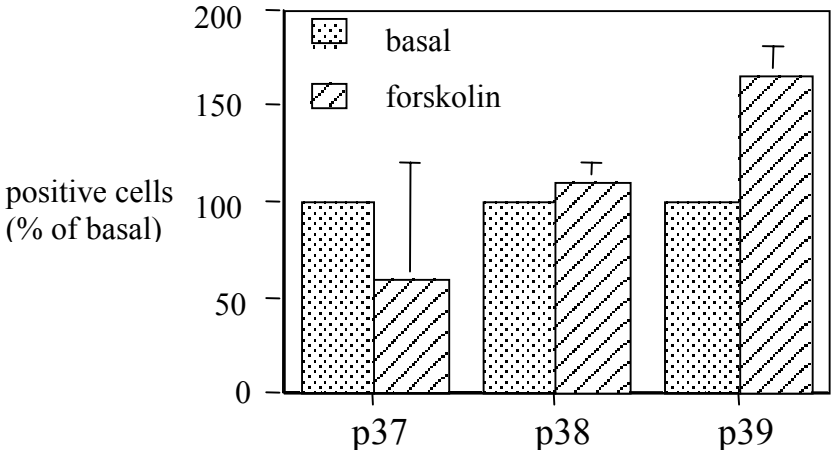
Supplementary Figure 1. PCR products from the Ura3 marker (right arm) and from the bacterial Amp-ori fragment (left arm) of the YAC, performed on the following samples: MC1 (CHO-MC1 cells, neg. control), yCFIRES (yeast cells, pos. control), P16, P37, P38 and P39. The size of the products is indicated to the right side of the figure. The presence of the expected bands for the left and right markers of the YAC suggest that the entire yCFIRES construct had been integrated into MC1.



Supplementary Figure 2. Quantitative PCR of P39 DNA samples with primers for CFTR exon 20 and actin gene. CFTR and actin band intensities were plotted against the amount of DNA present in each reaction. The data reported in the graphic refer to quantitation of 15 μl PCR aliquots.



Supplementary Figure 3. Expression of CFTR gene as determined by RT-PCR. The reactions were performed using primers spanning exon 7 to exon 9 (panel A) and primers spanning exon 11 to exon 13 (panel B) with the following RNA samples: MC1 (CHO-MC1 cells, neg. control), T84 (pos. control), P16, P34, P36, P37, P38 and P39. The presence of the expected band in all the clones suggested that CFTR was actively transcribed, although it is not possible to determine the level of transcription in the various clones.



Supplementary Figure 4. Immunocytofluorimetric analysis of the surface CFTR. CFTRp was revealed with an antibody to the first extra-cellular loop in cells nor fixed neither permeabilized. Cells were incubated in the presence of 5 μM forskolin or DMSO (basal) for 5 minutes at 37°C, washed, and analyzed. Data are expressed as percentage of DMSO-treated (basal) cells of 2-3 experiments (+/- SE).

METHODS

Fusion of CHO-MC1 cells with yeast protoplasts. Protoplasts from yeast cells containing yCFIRES (kindly provided by Dr. C. Huxley) were prepared for fusion according to Silverman *et al.* (1993). After protoplast formation the resulting cells were carefully washed in 1 M sorbitol and resuspended in STC (1 M sorbitol, 10 mM Tris base pH 8, 10 mM $CaCl_2$). One day before fusion CHO-MC1 cells were lipofected (Lipofectamine, Life Technologies) with 2 μg Sat2 DNA (Cooke and Hindley, 1979). At the time of fusion the cells were trypsined and washed three times in serum-free DMEM. $2x10^6$ cells and 10^8 protoplasts were mixed, centrifuged and resuspended in 500 μl of 50% PEG 1500 (Roche), 10 mM $CaCl_2$. The suspension was incubated for 90 sec and subsequently diluted with 5 ml serum-free DMEM, over a 5 min time period. After 30 min of incubation at room temperature, the cells were collected, resuspended in complete medium and distributed in multiwell plates. Selection with G418 was applied 48 h after transfection.

PCR and RT-PCR. PCR was performed in 25 μl reactions containing 100 ng DNA, 1X buffer, 1.5 mM MgCl2, 2 mM of each dNTP, 25 pmoles of each primer and 0.25 units Taq polymerase (Ampli-Taq from Perkin Elmer). After 5 min at 95 °C, the reactions were cycled 30 times at 95 °C for 1 min, 55-56 °C for 30-60 sec, 72°C for 30-60 sec. The RT-PCR reactions were performed with 300 ng RNA samples and Tth DNA polymerase (Roche) under the supplier's specifications.

CFTR heterologous competitor. The heterologous competitor was generated by PCR using the cftr exon20-trp1 hybrid primers and *Saccharomyces cerevisae* DNA. The primers were:

CF-trpF, TATATGTCACAGAAGTGATCCCATCACTTT*TTTCACAGGTAGTTCTGGTCC*

CF-trpR, TTCTGGCTAAGTCCTTTTGCTCACCTGTG*GCATTTTTGACGAAATTTGC*.

The 5' region of CF-trpF and CF-trpR consists of CFTR exon 20 sequences, forward and reverse respectively; the 3' region (in italics) consists of the yeast TRP1 sequence, forward and reverse respectively . The PCR product, after gel purification was cloned into pCR2.1-Topo (Invitrogen). The resulting plasmid (pComp) following linearization with *Not*I was used as a competitor.

Quantitative PCR. Limiting dilution PCR was carried out according to Schimtt, et al. (1996) with minor modifications. Genomic DNA was prepared by twofold serial dilution of the 50 ng/μl stock solution. For each experiment we prepared 12 PCR reactions containing 1 μl DNA from each dilution, primers for CFTR exon 20 and actin gene, in a total volume of 25 μl. Aliquots (5 and 15 μl) were separated on agarose gels, stained with ethidium bromide and quantitated by image analyzer (Kodak 1D). The intensity of the CFTR (213 bp) and actin bands (395 bp) was plotted against the amount of genomic DNA present in the reaction (Supplementary fig. 2). Within the exponential phase of the resulting line we determined CFTR/actin band intensity ratio. Other experiments were carried out taking into consideration the CFTR exons 13 and 10, each in combination with the actin. In all cases the CFTR/actin ratio was found to be similar with values of 0,5-0,6, confirming the presence of half target (CFTR) with respect to the standard.

Cytofluorimetric analysis. Cells were detached with 3 mM EDTA, incubated with MATG-1031 (1:200) in 2% FCS, 4% normal goat serum, 0.2% sodium azide for 30 minutes at RT, washed, and decorated with FITC-conjugated secondary anti-mouse antibody (1:200) for 30 minutes at RT. Cells were washed and analyzed by flow cytofluorimetry with a FACScan apparatus (Becton-Dickinson, San Jose, CA). As a negative control the cells were incubated in the absence of primary antibody. Signal obtained from MC1 was considered background and subtracted from each clone. To express the data, the cell number was plotted against the log of mean fluorescence intensity, with $1x10^4$ cells being measured at each determination.

Measurements of $^{36}Cl^-$ efflux. CHO cells were plated on 35-mm Petri dishes at a density of $3x10^5$ cells/dish and cultured for 3 days before $^{36}Cl^-$ efflux experiments. Cells were washed twice with standard saline solution containing: 130mM NaCl, 2mM KCl, 1 mM KH_2PO_4, 2mM $CaCl_2$, 2mM $MgCl_2$, 10mM Hepes, 10mM D-glucose, 20mM mannitol, pH 7.3 and incubated for 40 min at 37°C with 800 µl of the same solution containing 1 µCi $^{36}Cl^-$. At the end of the incubation, the loading medium was discarded, and cells were washed with ice-cold $^{36}Cl^-$-free medium. Efflux experiments started with the addition of 1 ml saline solution and every min, for a total of 7 min, the efflux medium was removed from the Petri dish and rapidly replaced by another ml of solution. The membrane-permeable cAMP analogue CPT-cAMP was applied at a concentration of 500 µM, 3 min after the beginning of the experiment and maintained up to the end of the experiment. At the end of the experiment, cells were lysed by incubation with 0.25 N NaOH. The radioactivity present in the efflux samples and in cell lysates was determined by liquid scintillation. Total incorporated $^{36}Cl^-$ (T_o) was

obtaining by adding the radioactivity in the efflux samples to that remaining in the NaOH extracts. $^{36}Cl^-$ remaining in the cells at a given time *t* (T_{res}) was determined by subtracting from T_o the amount of radioactivity in the efflux samples up to time *t*. The time course of $^{36}Cl^-$ efflux was expressed by plotting fractional efflux (FE) vs time. FE at a given time was calculated according to $FE= T_{ex}/T_{res}$, where T_{ex} is the amount of $^{36}Cl^-$ released in a single efflux interval and T_{res} is the amount of residual $^{36}Cl^-$ at the beginning of that interval. All experiments were done at 37°C.

Primers used in PCR and RT-PCR reactions

Ura3F	CCATCGATGGCCGTCGATGATGTGGTCTCTACAGG
Ura3R	TCTTCTCCAATATGCTTCCCAGCCTGC
Amp-oriF	ATGCCGGGAGCAGACAAGCCCG
Amp-oriR	GACCGCTGCGCCTTATCCGG
Exon7F	TGTGCTTCCCTATGCACTAAT
Exon9R	TCCTGTCCTGAAAGATATTAA
Exon11F	CTGAGTGGAGGTCAACGAGC
Exon13R	GCCTGAAGCGTGGGGCCAGT
β-actinF	TCAACACCCCAGCCATGTAC
β-actinR	AGGGCAGCGGAACCGCTCAT
β-actin2F	AACTGGAACGGTGAAGGCGAC
β-actin2R	GGGGCCACGATGGTTGACCA

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Schmitt, J.F. and Hearn M.T.W. (1996) The use of crude tissue section lysates for PCR assessment of gene copy number with human tumor biopsy samples. *J. Biochem.Biophys.Methods* **33**, 217-230.

Silverman, G.A., Yang, E., Proffitt, J.H., Zutter, M., Korsmeyer, S.J. (1993) Genetic transfer and expression of reconstructed yeast artificial chromosomes containing normal and translocated BCL2 proto-oncogenes. *Mol Cell Biol.*, **13,** 5469-78.