MAPPING THE BOVINE CYSTIC FIBROSIS GENE

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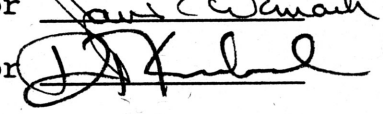
Honors Director 

Mapping the Bovine Cystic Fibrosis Gene

Abstract. Human cDNA probe H1.6 (clone 10-1) encoding cystic fibrosis transmembrane conductance regulator (CFTR) was used to map the bovine homolog of CFTR. Using a panel of bovine X rodent hybrid somatic cells, the homolog was mapped to bovine syntenic group U13. CFTR is 97.7% concordant with syntenic markers T-cell receptor beta (TCRB) and P-glycoprotein 3 (PGY3) previously mapped on U13. The comparative gene maps of CFTR, TCRB, and PGY3 on human chromosome 7, bovine syntenic group U13, and mouse chromosomes 5 and 6 indicate considerable evolutionary conservation.

Introduction

Cystic Fibrosis (CF) is the most common lethal human genetic disease affecting 1:2000 Caucasian and 1:17000 African-American births in the United States. CF is characterized by abnormal electrolyte transport that primarily affects the exocrine glands and respiratory system. CF is inherited in an autosomal recessive Mendelian fashion. The disease is caused by the production of a defective protein called the cystic fibrosis transmembrane conductance regulator (CFTR) which acts as a chloride conductance channel (Collins et al. 1992). The chief mutation that accounts for almost 70% of all CFTR mutations is a deletion of three base pairs that results in a lost phenylalanine at codon 508 (Kerem et al. 1989). The gene encoding CFTR has been mapped in humans to 7q31.3-32 (Rommens et al. 1989), and the murine homolog has been

cloned and assigned to mouse chromosome 6 (Siegel et al. 1992).

In the interest of comparative genome mapping between humans, mice, and cattle we have mapped the CFTR gene in cattle as described below.

Materials and Methods

Preparation of genomic DNA. The genomic DNAs of the bovine rodent hybrid somatic cell lines used in this study were prepared from the fusion of bovine peripheral leukocytes (BPL) with mutant rodent cell lines as described previously (Womack and Moll, 1986). The DNA samples were digested, electrophoresed, and blotted onto nylon membranes (Zetabind; AMF/CUNO, Meriden CT) according to established techniques (Sambrook et al. 1989).

cDNA probes. Initially two human DNA probes for CFTR were obtained from the American Type and Culture Collection (ATCC). Probe H1.6 (clone 10-1) cloned from human normal sweat gland epithelium was chosen because it was previously shown to hybridize with bovine DNA (Rommens et al. 1989). Clone T16-4.5 cloned from human colon carcinoma cell line T84 was also chosen based of its large size, 4.5 kb, which makes it a good candidate for hybridization to bovine DNA (Riordan et al. 1989). Upon further analysis of test blots containing five sets of genomic DNAs (rodent, bovine, and human) where each set was cut by a different restriction enzyme, clone 10-1 was selected to continue the mapping.

Plasmids were transformed and isolated according to standard

procedures (Ausubel et al. 1989). Plasmid inserts were further isolated by digestion with Eco RI and purified from agarose gel by electroelution. Probes were labeled with ^{32}p-dCTP to specific activities between 8.9 X 10^8 and 3.2 X 10^9 by the random primer method (Feinberg and Vogelstein, 1983).

A TCRB probe was genorously provided to our laboratory by Dr. H. Clevers and Dr. C. Baldwin (ILRAD, Nairobi). The TCRB probe contains only the D, J, and C region of cDNA derived from peripheral blood lymphocytes (Bensaid et al. 1989). This probe was also labled by the random primer method.

Southern Hybridization. The hybrid somatic cell DNAs were prehybridized at 42°C for 2-4 h in 5X sodium citrate, 10X Denhardt's, 0.05 M Na_3PO_4 (pH 6.7), 500 ug/ml sheared salmon sperm DNA, 5% dextran sulfate, 0.1% sodium dodecyl sulfate (SDS), and 50% formamide. Hybridizations were at 42°C for 18-20 h in 5X SSC, 1X Denhardt's, 0.02 M phosphate buffer (pH 7.0), 100 ug/ml sheared salmon sperm DNA, 10% dextran sulfate, 0.5% SDS, and 50% formamide. Washes to remove non-specifically bound probe were then made; two 15 min room temperature washes with 1X SSC and 1% SDS, and two 30 min 60°C washes with 1X SSC and 0.1% SDS. Filters were placed against Kodak XAR-5 film with one Dupont Cornex Lighting Plus intensifying screen (Dupont, Wilmington, DE) at -70°C for 1-5 days.

Assignment of CFTR to a syntenic group. A comparison of the retention and loss patterns of the CFTR fragment to previously

tested markers of each syntenic group was made and a percent concordancy calculated. CFTR was then assigned to the syntenic group to which it showed the highest level of concordance.

Results

Human DNA probe H1.6 clone 10-1 was hybridized to bovine X rodent hybrid somatic cell DNAs to determine the syntenic relationship of hybridizing sequences with previously mapped bovine genes. Resultant autoradiograms are presented in Figure 1.

From these data percent concordancies were calculated for markers on each bovine syntenic group (Table 1).

CFTR was assigned to syntenic group U13 with 97.7% concordancy to P-glycoprotein 3 (PGY3). Also, another marker for U13, TCRB (Li et al. 1992) was screened against the same panel of hybrid clones. Autoradiograms for TCRB probe can be seen in Figure 2. Again a concordancy of 97.7% is calculated between CFTR and TCRB.

Discussion

CFTR maps to HSA 7q 31.3-32 and bovine syntenic group U13. Other genes on U13 include PGY3, MET, and TCRB all of which map to HSA7, specifically 7q21, 7q31, and 7q35 respectively. MET and TCRB also map to mouse chromosome 6, but PGY3 maps to mouse chromosome 5 (Figure 3). Previous studies demonstrate conservation of genomic units in human, cattle, and mice, with more extensive syntenic conservation between human and cattle than between human and mouse (Womack and Moll, 1986; Threadgill and Womack, 1990a, 1990b;

Threadgill et al. 1991). The assignment of CFTR to U13 lends additional support to the hypothesis.

Mapping of the CFTR gene in cattle provides information for mammalian comparative gene mapping. While a bovine model would help in the advancement of a treatment for the human cystic fibrosis, naturally occuring animal models have not been identified. Currently, experimental therapy relies on the use of mouse models that have been created through homologous recombination (Snouwaert et al., 1992; Dorin et al., 1992).

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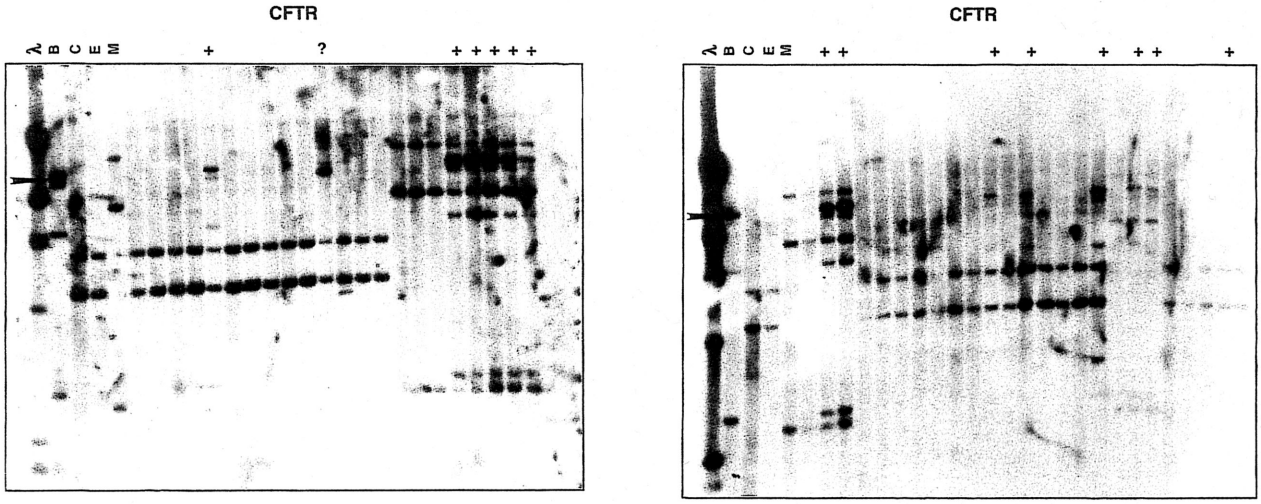

Figure 1. Southern blots of bovine (B), Chinese hamster ovary (C), E-36 (E), mouse (M), and hybrid somatic cells digested with Bgl II. The arrow points to a distinctive doublet in the bovine genomic DNA lane that can be used for scoring retention and lose patterns. Positive signs (+) indicate clones that retain CFTR. The question mark (?) indicates an ineffective restriction digest.

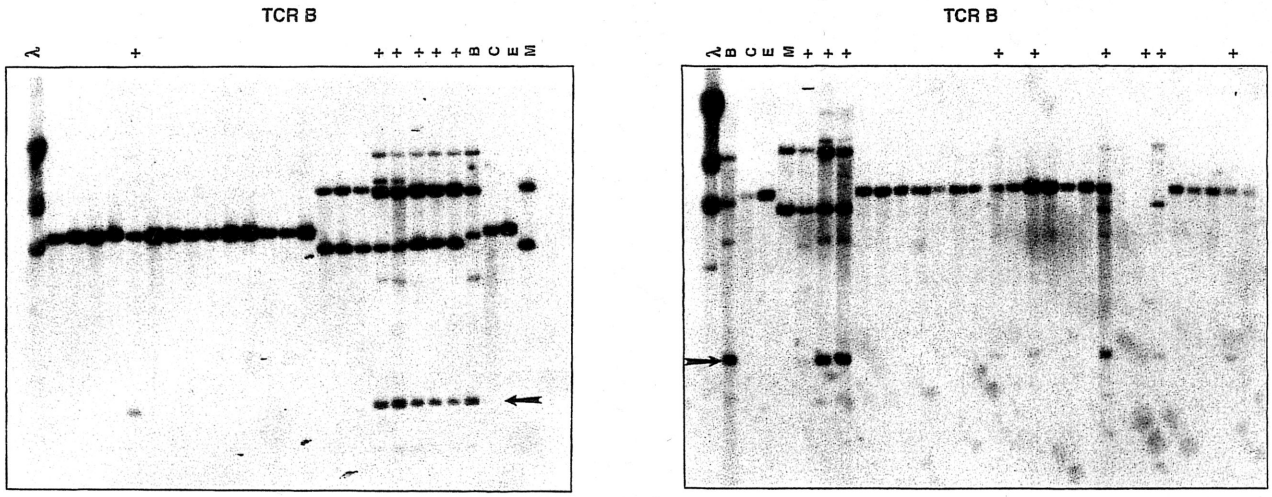

Figure 2. Southern blots of bovine (B), Chinese hamster ovary (C), E-36 (E), mouse (M), and hybrid somatic cells digested with Bam HI. The arrow points to a distinctive band in the bovine genomic DNA lane that can be used for scoring the retention and loss patterns. Positive signs (+) indicate clones that retain TCRB.

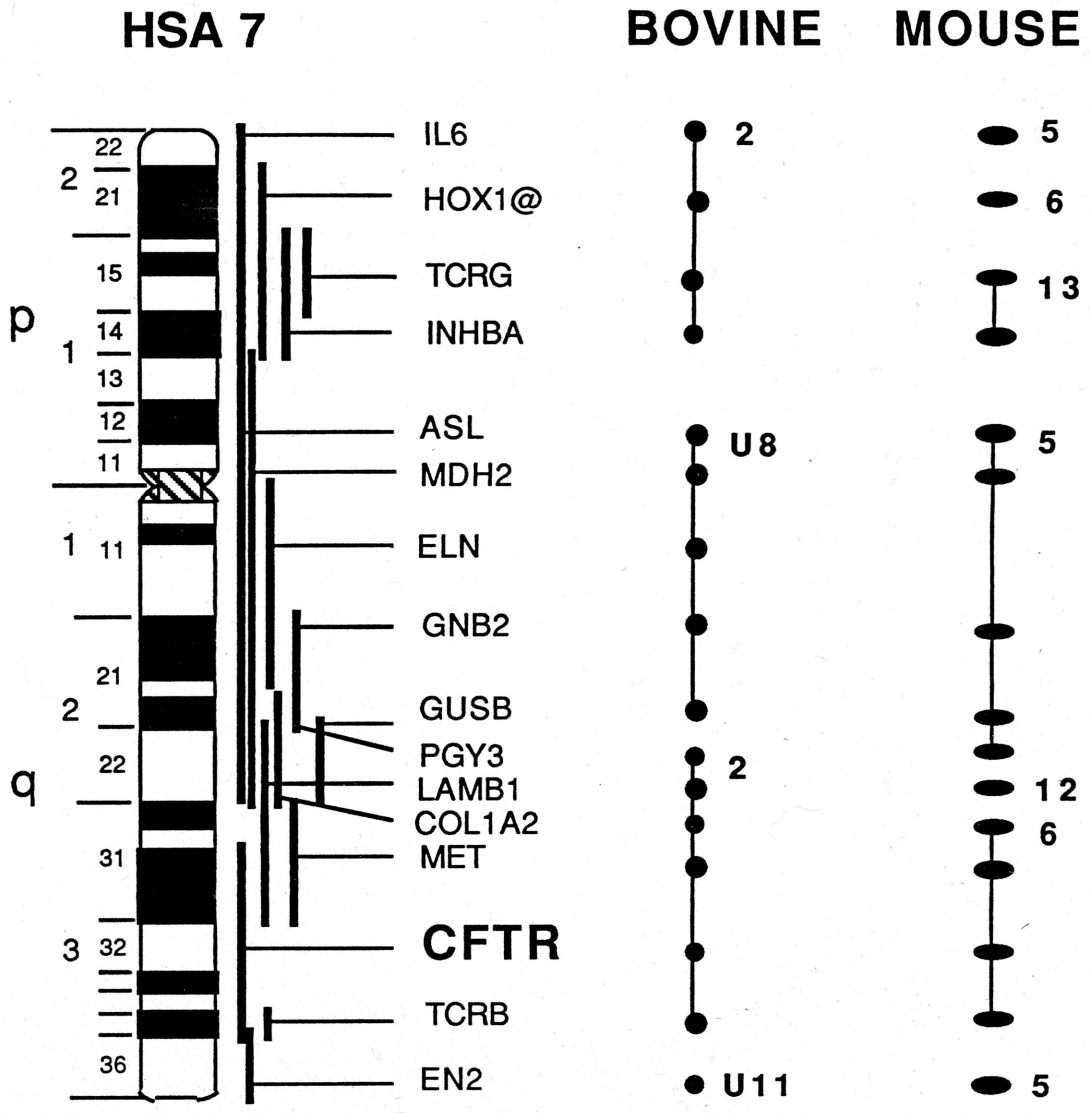

Figure 3. Comparison of chromosomal location of HSA7 loci in human, bovine, and mouse.

Table 1.

Concordancy of CFTR with bovine syntenic groups and marker genes

	%Concordancy CFTR
U1 (RDB23)	69.0
U2 (ESR)	73.0
U3 (IFNG)	60.0
U4 (RF131)	86.7
U5 (TCRA)	43.9
U6 (AMY1)	64.4
U7 (IGF2)	46.7
U8 (CD11A)	62.5
U9 (UPK1)	70.7
U10 (RDB21)	55.6
U11 (OXT)	50.0
U12 (GPX)	62.2
U13 (PGY3)	97.7
U14 (PLAT)	65.7
U15 (CASK)	66.7
U16 (POMC)	65.5
U17 (FUCA1)	77.8
U18 (IFNO)	73.3
U19 (CD3E)	51.1
U20 (PL)	65.9
U21 (PDEG)	61.4
U22 (INSR)	44.4
U23 (ALDH2)	73.3
U24 (TG)	61.4
U25 (ANT)	63.4
U26 (OAT)	56.8
U27 (F10)	64.4
U28 (MBP)	51.7
U29 (ACTA)	57.7

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