THE EXPRESSION AND CHARACTERIZATION OF HUMAN CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) IN TOBACCO

WILLIAM T. WITT

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Dr. Cynthia J. Denbow, Co-Chair

Dr. Carole L. Cramer, Co-Chair

Dr. Brenda S. J. Winkel

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(ABSTRACT)

The cystic fibrosis transmembrane conductance regulator (CFTR) is one of the most studied membrane protein models because of its clear clinical significance. Mutations within the CFTR gene lead to cystic fibrosis, the most common autosomal recessive genetic disorder in the Caucasian population. CFTR, a large 160 kDa glycoprotein, is a chloride ion channel in the ABC superfamily of transporter proteins. Due to low natural abundance of CFTR and difficulties producing sufficient amounts in heterologous systems, the exact protein function/structure relationship is unknown. Expression of CFTR in *E. coli* is lethal and mammalian culture systems are expensive and low yielding. However, successful bioproduction of many complex human proteins has been shown in transgenic plants. Our research objective is to develop tobacco as a model system for expressing human CFTR. Constructs of full-length CFTR fused to the 35S double enhanced promoter could not be propagated in *E. coli*, suggesting that the CFTR product generated by "leaky" expression was detrimental to bacteria. Two strategies were undertaken to address the problem: 1) a plant intron was introduced into CFTR sequence and 2) a more tightly regulated wound-inducible promoter MeGA^{TM} was used. Tobacco was transformed with all constructs. CFTR presence was determined by polymerase chain reaction (PCR). Expression and intron splicing was analyzed by reverse transcriptase-PCR. Splicing did not occur presumably due to intron /exon contexts. In tobacco expressing MeGA:CFTR, however, novel high-molecular-weight membrane-associated proteins were immunodetected using anti-CFTR antibodies suggesting that tobacco may be capable of producing human CFTR.

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List of Abbreviations

A	adenosine
ABC	ATP binding cassette
AMP	adenosine monophosphate
ATP	adenosine triphosphate
AU	adenosine uracil
bp	base pairs
BSA	bovine serum albumin
CaMV	cauliflower mosaic virus
CF	cystic fibrosis
CFF	cystic fibrosis foundation
CFTR	cystic fibrosis transmembrane conductance regulator
Cl^-	chloride ion
DE	dual enhanced
DEPC	diethylpyrocarbonate
DTT	dithiothreitol
EDTA	ethylene diamine tetra acetic acid
ENaC	epithelial sodium channel
ER	endoplasmic reticulum
GFP	green fluorescent protein
GUS	β-glucoronidase
HMGR	3-hydroxy-3-methylglutaryl coenzyme A reductase
kb	kilobases
kDa	kilo Dalton
LPG	lysophosphotidylglycerol
MeGA	mechanical gene activation
MRP	multidrug resistance-associated protein
MSD	membrane spanning domain
NPT	neomycin phosphotransferase
NBD	nucleotide binding domain
nt	nucleotides

PBS	phosphate buffered saline
PCR	polymerase chain reaction
PVP-40	polyvinylpyrrolidone
R	regulatory
RT-PCR	reverse transcriptase-polymerase chain reaction
SDS	sodium dodecyl sulfate
SSC	saline sodium citrate
T-DNA	transfer DNA
U	uracil