Construction of vectors for correction of the intestinal CFTR gene expression in Cystic Fibrosis sheep fetal fibroblasts

Kaden Bunch[1], Iuri Viotti Perisse[2], Zhiqiang Fan[2], Zhongde Wang,[2] Ann Harris[3], Kenneth L. White[2], Irina A. Polejaeva[2]

[1]*Department of Biology, Utah State University, Logan, Utah, USA.*

[2]*Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, Utah, USA.*

[3]*Department of Genetics and Genome Sciences, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA.*

Introduction

Cystic Fibrosis (CF) is a recessive human genetic disease that is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. This gene is responsible for transport of Cl^- and HCO_3^- anions in epithelial cells (Fig. 1).

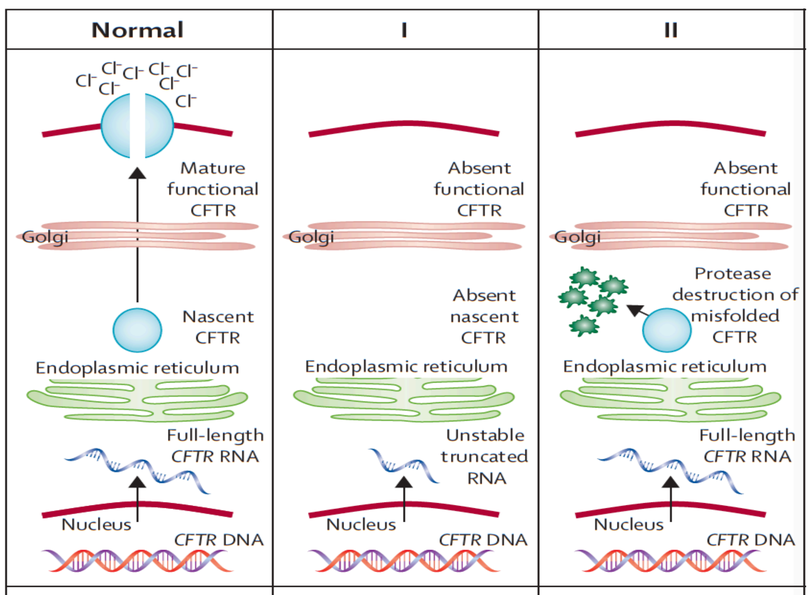

Figure 1. The CFTR expression in normal and mutated cells. Picture adapted from Elborn, 2016.

We previously generated *CFTR*$^{+/-}$ and *CFTR*$^{-/-}$ lambs using CRISPR/Cas9 and Somatic Cell Nuclear Transfer (SCNT) techniques (Fig. 2; Fan et al., 2018). The *CFTR*$^{-/-}$ lambs display many features similar to humans with CF, including meconium ileus (MI), pancreatic fibrosis, portal fibrosis and biliary hyperplasia, small gallbladder, and absence of the vas deferens. MI affects only 15-20% of human babies with CF; however, it was observed in 100% of newborn *CFTR*$^{-/-}$ lambs (Fig. 3) and was the primary factor that led to the euthanasia.

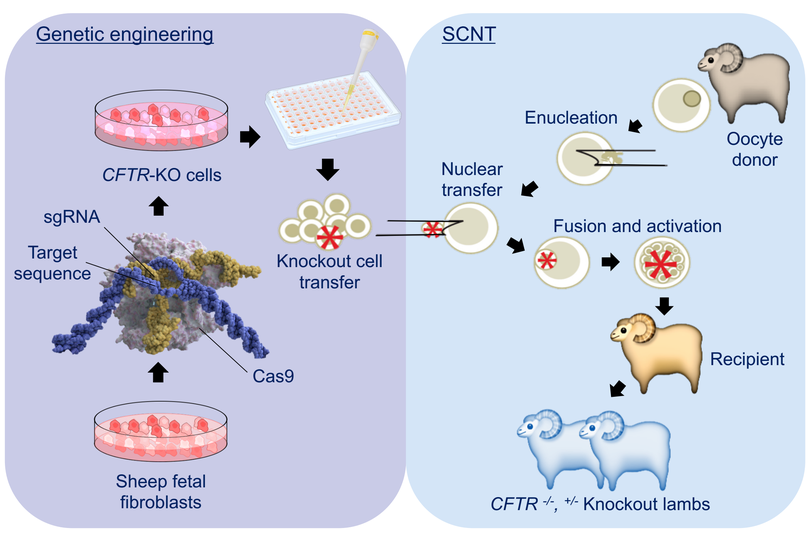

Figure 2. Production of *CFTR* -/-, +/- knockout lambs.

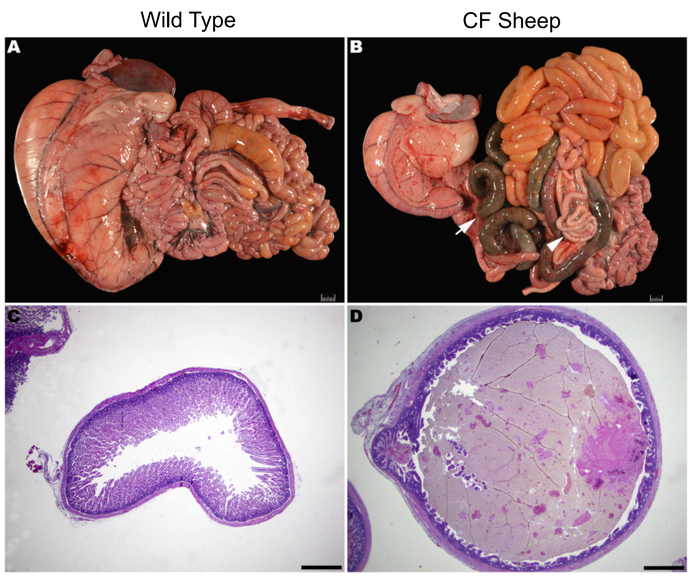

Figure 3: Lamb intestinal tracts and histology.

Objectives

- We hypothesized that the transgenic expression of ovine *CFTR* cDNA under regulation of an intestinal-specific promoter would promote the correction of MI in *CFTR*$^{-/-}$ sheep.
- In this study, we constructed three vectors with different intestinal promotors; the rat intestinal Fatty Acid Binding Protein (iFABP), rat liver Fatty Acid Binding Protein (LFABP), and Villin 1 promoters.

Methods

pcDNA3.1>*CFTR* vector constructions: The pcDNA3.1-CFTR-Zeo vector was a gift from Dr. Harris. The fragments (vector carrying the cDNA and the promoters) were digested and ligated into a single vector using cloning techniques. The vectors size: 10.6 Kb for iFABP, 9.8 Kb for LFABP, and 22 Kb for Villin (Fig. 4).

PCR analysis: Primers flanking the junction (i.e. promoter and cDNA) were designed and amplified by PCR to confirm the presence of the promoter sequences in the vectors (Table 1). The bands were analyzed using 1% agarose gel electrophoresis (Fig. 5 and 6).

Sequencing: PCR products were analyzed by Sanger sequencing (Fig. 7).

Results

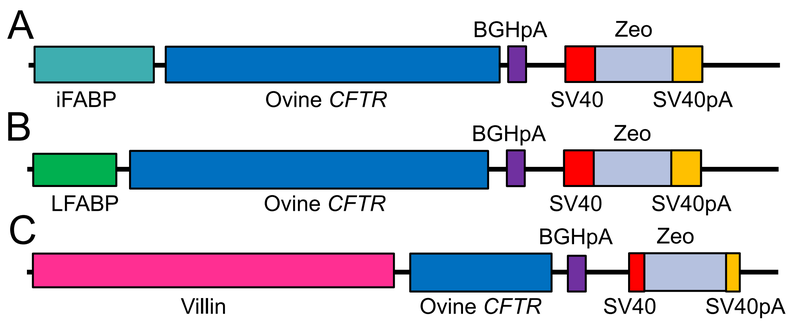

Figure 4. pcDNA3.1>*CFTR* vector design. (A) Vector with iFABP promoter. (B) Vector with LFABP promoter. (C) Vector with Villin promoter.

Table 1: Primers used in the identification of colonies by PCR amplification.

Name	Sequence (5'->3')	PCR product size
iFABP J2 F	AGAAACTAAAGGGGCCTGGCAT	527 bp
LFABP J2 F	ACTTCTGCCTTGCCCATTCTGA	551 bp
Villin J2 F	GATCATCATCAAAGCCGGGTGG	530 bp
CFTR J2 R	TTGAAGCCAGTTCTCTGTCCCA	-

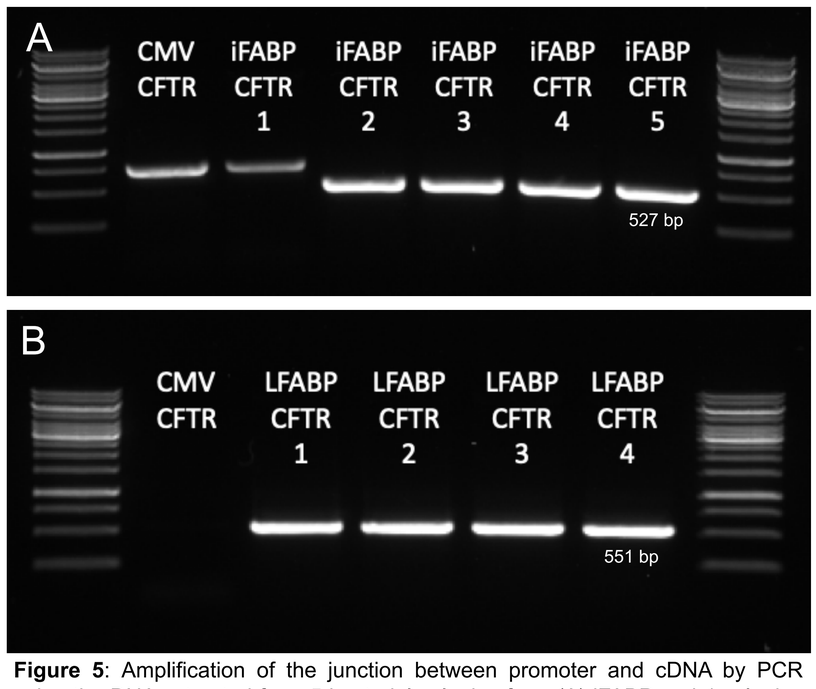

Figure 5: Amplification of the junction between promoter and cDNA by PCR using the DNA extracted from 5 bacterial colonies from (A) iFABP and 4 colonies from (B) LFABP vectors.

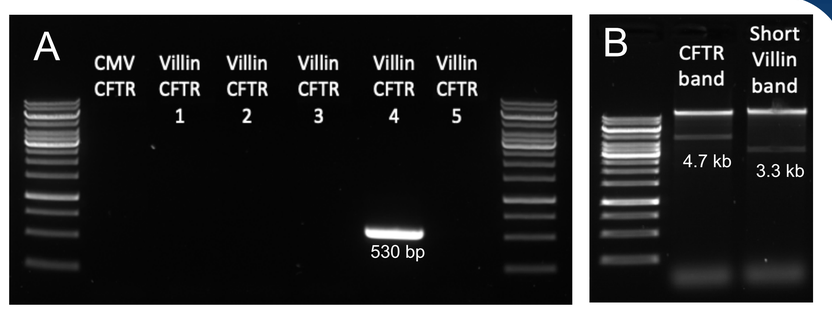

Figure 6: (A) Identification of Villin-CFTR bacterial colonies by PCR amplification. (B) Identification of CFTR cDNA and partial Villin promoter fragments.

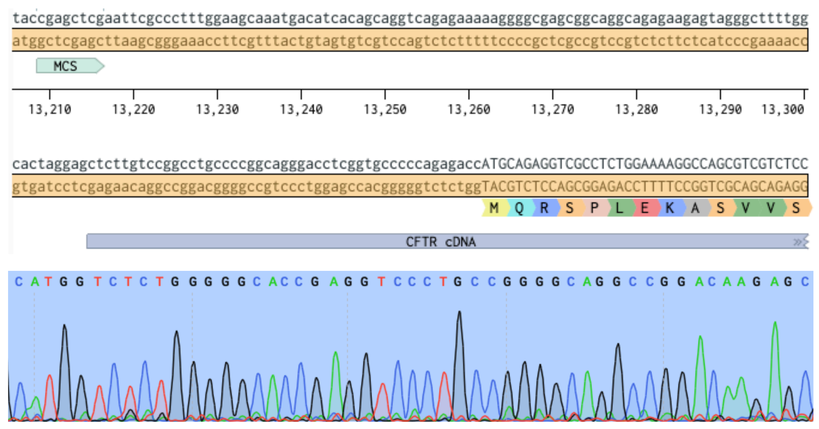

Figure 7: Sequencing of the junction region between Villin and CFTR.

Our results indicate that the transgenic vectors containing the ovine CFTR were successfully constructed under the regulation of either rat iFABP, LFABP, or Villin-1 promoters. For each construct, five bacterial colonies were isolated for identification. The PCR products were extracted from gel and subsequently Sanger sequencing analyzed to confirm that four iFABP colonies (Fig. 5A), four LFABP colonies (Fig. 5B), and one Villin colony (Figure 6A) were correct. It can be noted that the success rate of the ligation was far greater for the LFABP and iFABP promotor regions as compared to the Villin promoter.

Summary

- We accomplished the construction of the expression vectors containing the *CFTR* cDNA under regulation of iFABP, LFABP, and Villin.

Ongoing work

- We are assessing iFABP, LFABP, and Villin protein expression in sheep timed pregnancies by ImmunoHistoChemistry to determine which promoter would be most effective.
- We will evaluate the transient gene expression of the constructs in CaCO-2 cells to ensure they are fully functional.
- Production of single-cell SFF derived colonies containing integrated vectors.
- The selected colonies will be used in SCNT for production of "gut-corrected" CFTR-/- lambs.
- Evaluation of the phenotypes of CF lambs for the presence of meconium ileus.

References

1. Elborn, J. S. (2016). Cystic fibrosis. *The lancet*, *388*(10059), 2519-2531.
2. Fan, Z., I. V. Perisse, C. U. Cotton, M. Regouski, Q. Meng, C. Domb, A. J. Van Wettere, Z. Wang, A. Harris, K. L. White and I. A. Polejaeva (2018). "A sheep model of cystic fibrosis generated by CRISPR/Cas9 disruption of the CFTR gene." JCI Insight **3**(19).

Acknowledgements

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