

Two major repression mechanism of utrophin expression: Potential Therapeutic Avenues for DMD

¹Utpal Basu, ¹SK Tousif Ahamed, ¹Debasish Malik

¹Dept. of Molecular Biology and Biotechnology, University of Kalyani, Kalyani, Nadia, India. email utpal@klyuniv.ac.in, utpal_basu@yahoo.com
Funding partner Parent Project Muscular Dystrophy-India, matex1884@gmail.com, info@ppmdindia.org Tel +91 9870304838, www.ppmdindia.org

Abstract: We have identified two major repression mechanisms of utrophin expression : (i) a small sequence element that contributes in its post-transcriptional repression and (ii) nuclear retention of utrophin mRNA. Both the mechanisms operate in mouse myoblast C2C12 cells. The repressor sequence is highly conserved between human and mouse, and the RNA-seq analysis supports nuclear retention of utrophin mRNA in mouse muscle (Ghosh et al RNA Biology (2021)). Now we present evidence of nuclear retention of utrophin mRNA in human 293T cells.

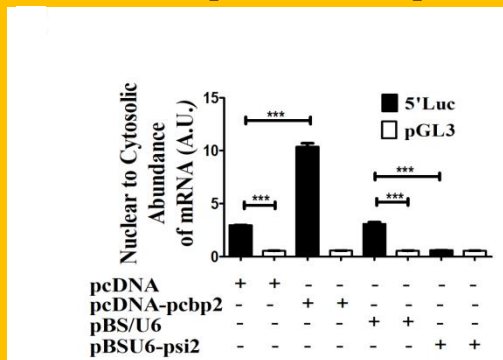
DMD & Utrophin



✦ Utrophin, the autosomal homologue of dystrophin can compensate dystrophin deficiency in animal models

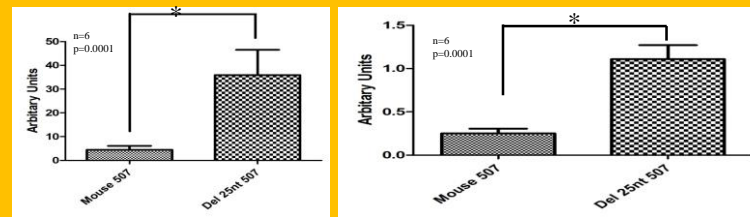
✦ Upregulation of indigenous utrophin does not evoke immune response in animal models.

507 nt 5'UTR in 5'Luc reporter plays key role in PCBP2 mediated nuclear retention of reporter transcripts



Ghosh et al. RNA Biology (2021)

Dual luciferase assay of reporter plasmid & mRNA transfection



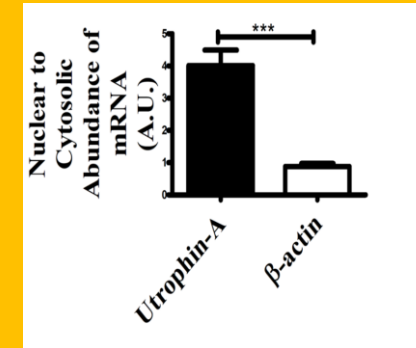
WT construct



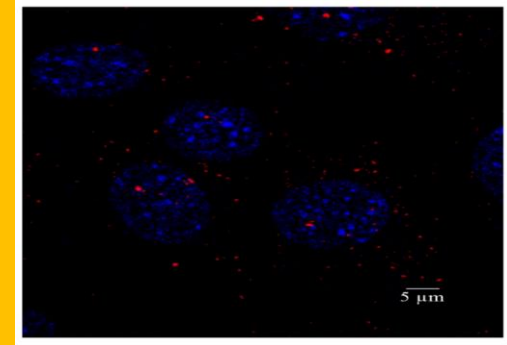
Δ25nt construct



Retention of mouse utrophin-A mRNA in the nucleus



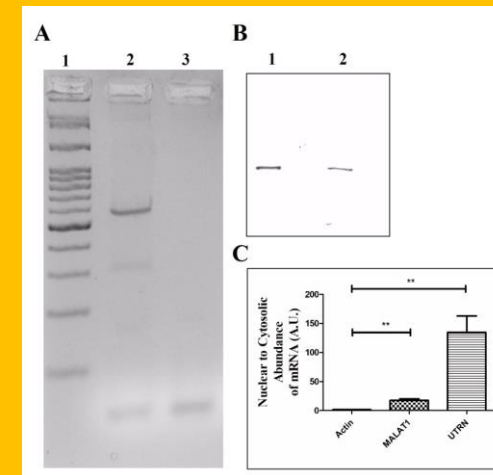
NF vs CF abundance of utrophin mRNA in non-transfected C2C12 cells



RNA FISH of utrophin in non-transfected C2C12 cells

Ghosh et al. RNA Biology (2021)

Nuclear retention of utrophin mRNA in 293T.



(A) The RT PCR of human utrophin-A mRNA with primer pair specific for 590 nt long 5'UTR. Lane 1: 100 bp ladder; lane 2: cDNA from 293 cells; Lane 3: (-)ve control. (B) Western blot analysis of C2C12 and 293 lysate with anti utrophin antibody. (C) Nuclear to cytosolic abundance of UTRN transcript as determined by qPCR from the nuclear and cytosolic fractions of 293T cells. The result is plotted as mean ± SD. ** p < 0.01 (Student's t-test)