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An upstream open reading frame and two *cis*-acting sequence elements in mouse utrophin-A 5'-UTR repress cap-dependent translation

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Duchenne Muscular Dystrophy (DMD) is the most common X-linked genetic disorder that occurs 1 in every 3500 male child birth worldwide. There is no strategy available to cure the fatal disease. DMD results from the mutation in the dystrophin gene that leads to formation of non-functional or truncated dystrophin. Dystrophin is the integral component of dystrophin associated protein complex which links between actin filament and extracellular matrix and therefore maintains sarcolemmal integrity in muscle fiber. Up-regulation of utrophin, the autosomal homologue of dystrophin has been suggested as one of the promising strategies for DMD treatment. In adult utrophin expression however is confined to neuromuscular and myotendinous junction. Therefore understanding the regulation of utrophin expression has therapeutic importance. Although the transcriptional regulation of utrophin-A, the skeletal muscle specific isoform has been well studied, limited works have been focused on its post-transcriptional regulation. Previous studies suggested an Internal Ribosome Entry Site (IRES) in utrophin-A 5'-UTR. Repression of translation has also been demonstrated with this 5'-UTR. In order to improve the understanding, the present study compared contribution of cap-dependent as well as cap-independent translation with utrophin-A 5'-UTR with m7G and A-capped RNA transfection based approach. Compared to well studied encephalomyocarditis virus IRES, cap-independent translation with utrophin-A 5'-UTR is weak. However, its contribution becomes significant as cap-dependent translation is severely repressed with it. We further identified two cis-acting elements and one upstream open reading frame in utrophin-A 5'-UTR responsible for translation repression. The repressor elements may be targeted for up-regulation of utrophin-A expression.

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Identification of a novel hepatocyte nuclear factor-1 transcription factor binding site on polycystic kidney disease-1 gene promoter

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Human Polycystic Kidney Disease-1 (pkd1) gene promoter possesses various transcription factors in its vicinity which regulate its activity. The regulatory processes thus, layout the role of various transcriptional elements associated with the pkd1 gene in understanding the Autosomal Dominant Polycystic Kidney Disease (ADPKD) pathophysiology. These transcription factors either coalesce through coordinated interaction or work independently of each other hence maintaining pkd1 gene expression. There are substantial numbers of transcription factors which still remain to be identified. Identification of human pkd1 gene mutations within 5' untranslated region together with haplo-insufficiency has shifted the focus towards understanding gene regulatory pathways at pkd1 gene locus. As a result, 3.3-kb, -2.0-kb, -0.95 and -0.2-kb 5' proximal regions of the human pkd1 gene promoter were analyzed to posses Hepatocyte Nuclear Factor-1 (HNF-1β) transcription factor binding site. The results show that HNF-1β co-localized with P300/PCAF in nucleus and up-regulates pkd1 gene regulation. Deletion construct analysis revealed presence of HNF-1β consensus sequences in the -0.95-kb region and posses a functional binding site through histone acetyl-transferase (p300/PCAF). Localization studies also confirmed its subcellular location to the nucleus using GFP with DAPI stained nuclei expressed in HEK293T cells. RT-PCR showed that overexpression of HNF-1β treatment of HEK293T cells increased the levels of endogenous pkd1 RNA and EMSA and chromatin immunoprecipitation showed the presence of a novel HNF-1β binding site at the pkd1 proximal promoter other than the typical evolutionary binding site. Therefore, these transcriptional studies and various molecular cross-talks bring out the mode of understanding ADPKD targeting the mechanism associated with HNF-1β up-regulation.

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