March 1999 (Volume 40, Number 4)

Premature Termination Codon in the Aggrecan Gene of Nanomelia and Its Influence on mRNA Transport and Stability

Dragan Primorac[1,3], *Carol V. Johnson*[2], *Jeanne B. Lawrence*[2], *Monique B. McKinstry*[3], *Mary Louise Stover*[3], *Moses S. Schanfield*[4], *Šimun Anðelinoviæ*[1], *Tade Tadiæ*[1,3], *David W. Rowe*[3]
[1]Split University Hospital, Laboratory for Clinical and Forensic Genetics, Split, Croatia; [2]University of Massachusetts, Department of Cell Biology, Worcester, Mass; [3]Department of Pediatrics, University of Connecticut, Farmington, Conn; and [4]Analytical Genetic Testing Center, Denver, Colo, USA

Aim. To analyze the influence of the premature termination codon on mRNA transport and stability.
Methods. Chondrocyte mRNA was isolated from homozygous and heterozygous nanomelic 17-days old embryos and examined by RT-PCR analysis. To analyze aggrecan mRNA stability, mRNA synthesis was inhibited with DRB [5,6 dichloro-1-(-D-ribofuranosyl benzimidazole)], a specific inhibitor of RNA polymerase II. Visualization of the aggrecan alleles was performed by in situ hybridization.
Results. The level of mutant aggrecan mRNA within the nucleus was equal to that of the control, but no mutant mRNA was observed in the cytoplasm. RT-PCR revealed that the mutant transcript was only detectable in the nucleus, compared with house-keeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene or collagen type II. A restriction site induced by premature termination codon TAA allowed the distinction of normal and mutant transcripts in chondrocytes derived from embryos heterozygous for the nanomelic mutation. After the treatment with DRB, identical decay rates were demonstrated for both transcripts within the heterozygous nucleus. In situ hybridization showed no abnormal mRNA accumulation.
Conclusion. This is the first evidence suggesting that the transcript of the mRNA with the premature termination codon within an exon does exit the nucleus.

Key words: chondrocytes; codon, stop; codon, terminator; collagen type II; mRNA; peptide chain termination; RNA, messenger; translation, genetic

Copyright © 1999 by the **C**roatian **M**edica**l** **J**ournal. All rights reserved. Created 1/12/99 - Last Modified 1/12/99
Created and maintained by: Tinman