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Reply to Inácio *et al*

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We have recently reviewed the physiological role of nonsense-mediated decay (NMD) and its implications for human genetic disorders. The goal of our review paper was to make the medical community aware that mRNA processing steps must be taken into consideration when attempting to elucidate genotype–phenotype relationships.[1] Although the exact mechanism for NMD remains to be determined, previous studies in model organisms provide insight into how transcripts containing premature termination codons (PTCs) are recognized by NMD factors and rapidly degraded resulting in loss-of-function alleles.[2] Evidence from a number of studies suggests a model for the mechanism of NMD surveillance system in which a PTC in the last exon, or within less than ~55 base pairs from the final intron in penultimate exon (also known as '55 nt boundary rule'), is not recognized and may cause expression of large amounts of aberrant truncated proteins with potential harmful effects in cells. We also review and cite the observations that several genes appear to show exception to this rule and thus, mRNAs with nonsense codons present in such positions are subject to the NMD pathway. This suggests either additional or entirely different *cis*-acting signals may exist to initiate NMD for select genes.[3]

NMD mechanistic models will require refinement with additional experimental observations as more genes (eg *HBB* for human β-globin and *BRCA1*) are examined. Recent studies have shown that premature nonsense mutations located proximal to an internal AUG in some genes may escape NMD and result in synthesis of N-terminally truncated proteins by a 'translation re-initiation' mechanism.[4–6] The extent of the effect of escaping NMD by this mechanism and its implications for genotype–phenotype correlations in human disease may also vary depending on both the toxicity of truncated proteins and the nature of traits encoded by different genes. These observations define another factor that can contribute to the complexity and efficiency of the NMD surveillance system in regulating gene expression.

Nevertheless, these findings further support the thesis of our review that one must recognize the role of mRNA and protein processing steps (ie information transfer downstream from the DNA mutation) in conveying the ultimate clinical outcome. It also illustrates the view that understanding of the molecular mechanism of NMD is far from complete and there are no 'universal rules' that have been defined for all genes. Thus, it is essential to carefully verify the potential role of NMD experimentally to enable accurate conclusions regarding genotype–phenotype correlations underlying genetic disorders.

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