ABSTRACT

Nonsense-mediated mRNA decay (NMD) is a translation-dependent surveillance mechanism that controls gene expression by degrading different classes of target transcripts with premature or aberrant translation termination. Besides its function in clearing the cell of erroneous transcripts, NMD also regulates a variety of wildtype messenger RNAs (mRNAs). Thereby NMD shapes the transcriptome of different cell types, tissues and even individuals. Moreover, NMD is implicated in the modification of various disease phenotypes.

Target degradation is initiated by the central NMD factor UPF1 that recruits the NMD effectors SMG6 and the SMG5/7 heterodimer. Two decay routes are majorly involved in substrate decay: SMG6-mediated endonucleolytic cleavage and deadenylation-dependent decapping induced by SMG5/7. The extent to which these decay routes contribute to the degradation of individual transcripts is poorly understood.

Endonucleolytic cleavage, that segregates the mRNA into a 5′ and a 3′ fragment, is considered the main degradation pathway during NMD. However, the exact sites of endocleavage remain to be determined and most evidence for SMG6-specific substrates is indirect. In this work, a method was established that enables the direct identification of endogenous NMD targets by transcriptome-wide mapping of 3′ decay intermediates. Investigating the sites of endocleavage, globally and at single nucleotide resolution, reveals that endocleavage occurs at various NMD-inducing features, including premature translation termination codons (PTCs), upstream open reading frames (uORFs), selenocysteine codons (Sec codons) or long 3′ untranslated regions (3′ UTRs). Furthermore, the interplay between endocleavage and deandenylation-dependent substrate decay was investigated. It was observed that SMG7 can regulate the endocleavage efficiency of NMD substrates with long 3′ UTRs, uORFs and Sec codons but does not affect PTC-containing transcripts.

Taken together, the findings of this work indicate that SMG7 is able to regulate SMG6-mediated endocleavge of several target transcripts and that the extent to which SMG7 can influence endocleavage efficiency is determined by the mRNA architecture and the composition of RNA-binding proteins. Thereby, NMD can exert degradative and regulatory

functions on the transcriptome, underlining its physiological function in globally modifying gene expression, in addition to the degradation of faulty transcripts.