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Simplified Nonsense: New Methods for Interrogating NMD

Hanna Alalam
Institutionen för kemi och molekylärbiologi
Naturvetenskapliga fakulteten

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ABSTRACT

Nonsense mediated decay (NMD) is a pathway that regulates RNA turnover. Since its discovery, this pathway has been implicated in a variety of cellular processes ranging from differentiation to the restriction of viral replication. While NMD has been heavily studied since its discovery, the understanding of how the pathway carries out its function has been a long and convoluted process, where the current cornerstones that establish our present understanding of the regulatory mechanisms are continuously challenged.

In this thesis, new methods were explored with the goal to provide tools that would simplify investigating the NMD pathway and potentially other pathways regulating RNA. We studied the use of nucleotide conversion methods and their applicability to yeast. Additionally, we designed a set of reporters that allow *in vivo* monitoring of NMD with an easy-to-read phenotype as an output. Moreover, we modified a reporter that was developed during the construction of the NMD reporters to also be applicable for alternative studies. In this particular case, we adapted one of our reporters to the study of the SARS-CoV-2 major protease (NSP5). Overall, simplified methods to interrogate cellular NMD were successfully constructed, in addition to establishing a sensitive yeast-based system for the detection of anti-viral compounds.

Keywords: mRNA degradation, metabolic labeling, nonsense-mediated decay, Major protease, SARS-CoV-2