Abstract

Nonsense-mediated mRNA Decay (NMD) is an mRNA quality-control mechanism that selectively recognizes and degrades aberrant transcripts harboring a premature termination codon (PTC), which could result from DNA mutation or from errors occurred during RNA metabolism. NMD could prevent the production of deleterious C-terminal truncated proteins (Chang et al., 2007). The highly conservation of NMD pathway through evolution suggests that it could play a more relevant biological role in the post-transcriptional regulation of gene expression rather than just degradation of PTC-harboring mRNAs. Indeed, many eukaryotic genes are regulated by the association between alternative splicing and NMD (AS-NMD): the alternative splicing would produce an unproductive mRNA isoform that harbors a PTC; NMD, which degrades the aberrant transcript, may modulate the RNA levels and, thus, the corresponding protein amount in the cell. Ribosomal protein genes represent a good model to study post-transcriptional regulation by AS-NMD. Ribosomal protein L3 (rpL3) pre-mRNA can undergo a canonical splicing, which produces an isoform properly translated into protein, or an alternative event, that produces a PTC-harboring isoform eliminated by NMD. It was demonstrated that free rpL3 protein (i.e. the ribosomal protein not involved in ribosome formation) regulates the rpL3 pre-mRNA splicing. In physiological condition, canonical splicing prevails over the alternative event. When there is an excess of rpL3 protein in the cell, the alternative splicing event is favoured, thus leading to a decrease of protein amount (Cuccurese et al., 2005). The aim of this project is to clarify the molecular mechanism of the post-transcriptional regulation of rpL3 gene through the splice site selection that leads to canonical or alternative isoform production. Since it was demonstrated that rpL3 is not able to interact directly with its pre-mRNA, rpL3 protein and pre-mRNA interactors in vitro were identified. Among these, I have investigated the role of two splicing factors, heterogeneous nuclear RiboNucleoProtein H1 (hnRNP H1) and K-homology splicing regulator protein (KHSRP), and of the nucleolar phosphoprotein Nucleophosmin/NPM on the rpL3 pre-mRNA splicing. These three proteins are involved in several phases of mRNA processing.