Title
Two degrading decades for RNA

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Two degrading decades for *RNA*

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The last 20 years have seen an explosion of discoveries in the field of RNA research. Having obtained my PhD working on group II intron self-splicing, and given the mechanistic relationships between these introns and spliceosome-mediated splicing, I feel that one of the most remarkable achievements in the last two decades was the demonstration that RNA catalyzes splicing within the spliceosome, further strenghthening the mechanistic similarities between these splicing systems. After getting my PhD in 1995, one year after the birth of the *RNA* journal, I focused my interests on the mechanisms of mRNA processing and degradation in eukaryotes, and witnessed in the last 20 years the amazing advances in identifying molecular pathways involved in RNA decay. For many molecular biologists, RNA interference (RNAi) has emerged during this period as the most popular mechanism of RNA degradation, as the discovery of RNAi opened the door to performing genetic loss of function studies in many organisms for which this type of study used to be particularly cumbersome. However I like to think that RNAi has been the tree that hides the forest of RNA decay, as that the last 20 years of research have brought us tremendous insights into the variety of molecular pathways and complexes that target and degrade eukaryotic RNAs. I will highlight below some of the most important discoveries in this field within this period, with apologies in advance for presenting a biased view and non-comprehensive overview, and for not giving credit to the authors who performed these studies. I also thank all my mentors, colleagues and mentees for having provided me with the opportunity to work in the RNA field during these last 20 years, and for sharing their exciting discoveries with me.

We have seen some major advances in our understanding of the basic mechanisms of eukaryotic mRNA decay. Several seminal studies have shown that deadenylation followed by decapping is responsible for the bulk of cytoplasmic mRNA degradation, as this pathway makes the mRNA susceptible to degradation by the 5′-3′ exonuclease Xrn1, by removing the protective 5′-cap. Interestingly, degradation by Xrn1 was found to occur within the context of translating ribosomes, providing a mechanistic explanation as to why translation destabilizes mRNAs. We have also learned that degradation from the 3′-end is a major mechanism to degrade eukaryotic mRNAs. The discovery of the exosome complex of 3′-5′ exonucleases provided us with one of the most complex degradative machines, which targets a large variety of RNAs in the nucleus and the cytoplasm. Strikingly, it was discovered by several groups that the addition of a short poly(A) tail to the 3′-end of RNAs by a specific class of poly(A) polymerases strongly enhances the degradative activity of the exosome. This discovery was a major breakthrough in the field as it showed that poly(A) addition can trigger RNA degradation in eukaryotes, as it does in bacteria, and in contrast to its traditional role as a mechanism to stabilize mRNAs and enhance export and translation. Fifteen years after its discovery in 1997, we now have several atomic resolution structures of the exosome, which have brought tremendous insights into the mechanisms of degradation by this remarkable molecular machine. Finally we now know that the exosome targets a large variety of transcripts in the cell, from precursors of noncoding RNAs for processing purposes to RNAs that are defective and need to be eliminated, introducing the concept of RNA quality control (QC).

Indeed, the last two decades have also seen major advances in the identification of QC pathways for many classes of RNAs. Many mRNAs are produced with defects during their synthesis or get stuck in the act of translation, and these RNAs need to be degraded through specific QC mechanisms. Twenty years ago, nonsense-mediated decay (NMD) was the major QC mechanism known to couple aberrant translation termination—i.e., recognition of a premature stop codon—to degradation of these so-called defective RNAs in eukaryotes. The mechanism of NMD has become clearer in the last two decades, and we understand better how NMD distinguishes a premature stop codon from a normal one. The discovery of the exon–exon junction complex was a major breakthrough for this problem, as it was determined that the distance from the stop codon to the last exon–exon junction complex plays a major role in deciding whether or not to send the mRNA through the NMD pathway. In addition, it was discovered that recognition of premature stop codons was found to

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occur during the pioneer round of translation. However the determinants of NMD are complex, since it was established that the distance from the stop codon to the poly(A) tail can also play a role depending on the model system.

Strikingly, it was found that other defects during the translation cycle can also trigger ribosome dissociation and RNA degradation. Messenger RNAs which are stalled in translation were found to be discarded by an endonucleolytic pathway, No-Go Decay (NGD); mRNAs lacking stop codons were found to be degraded by Non-Stop Decay (NSD), a cytoplasmic process which involves the cytoplasmic exosome. mRNAs subject to NGD could arise from blocks in translation due to RNA structures that block elongation, rare codons or chemical and oxidative damage to RNA, while mRNAs engaged in NSD could have resulted from defective polyadenylation within open-reading frames. This concept of RNA QC is not limited to mRNAs that trigger defects of translation, as several seminal studies have shown that various noncoding RNAs also undergo extensive QC. Defective tRNAs were reported to be degraded through a variety of QC mechanisms, including tagging by polyadenylation and degradation at the 3′-end by the nuclear exosome, as well as degradation from the 5′-end through the rapid tRNA decay pathways. Ribosomal RNAs that are defective for translation were also found to be subject to a specific discard mechanism, the nonfunctional rRNA decay (NRD) pathway. Interestingly some specific factors involved in NRD also play a role in NGD, leading to the idea of functional convergence of mRNA and ncRNA QC pathways.

During the last two decades, our understanding of the impact of RNA degradation on gene expression has also evolved rapidly from local to global. While papers from the late 1990s through the early 2000s focused mainly on the impact of RNA degradation pathways on model transcripts or reporters, the field has progressively moved towards genome-wide studies and it is now typical for research articles to include an assessment of the impact of these pathways at the genomic scale. Initial genome-wide studies relied on the use of cDNA or oligonucleotides based microarrays, and these technologies had their decade of glory from the mid-1990s through the end of the 2000s. During the last seven years, high throughput sequencing has progressively displaced microarrays as the technique of choice for identifying RNAs impacted by loss of function of specific RNA degradation pathways. In the meantime, experimental approaches have also evolved and become more refined. Many early genome-wide studies relied on assessing the effect of gene knockouts or conditional alleles/depletion on assessing transcript levels. While these studies provided valuable information, many of the variations observed were due to indirect effects; measurement of RNAs half-lives in the absence of specific degradative factor was a major step forward in the identification of specific targets. In the recent years, techniques involving protein–RNA cross-linking followed by immunoprecipitation and high throughput sequencing have provided a complementary approach to identify direct targets of RNA binding proteins, including many degradation factors.

The next two decades will likely provide us with more insights into the mechanisms of various RNA degradation pathways and how these pathways affect the transcriptome not only of model organisms, but also in a variety of non-classical models. A surprising recent finding was that some RNA degradation factors seem to directly impact transcription, leading to a more complicated understanding of the impact of degradative factors on gene expression, and to the concept of buffering in gene expression. Future studies will determine the molecular basis for the mechanistic relationships between transcription and RNA degradation. There have also been suggestions that RNA decay might occur in specialized subcellular dynamic structures, but the overall contribution of these structures to decay is still controversial and remains to be clarified. In addition, while the identification of NGD, NSD and NRD delineated new RNA QC pathways, we still lack a full understanding of the molecular mechanisms that govern these pathways and of the factors involved. We also still do not know how many natural mRNAs are subject to NGD and NSD. It is clear that RNA QC play essential functions in shaping the transcriptome and in controlling cellular functions in eukaryotes, as defects in NMD factors result in intellectual disability, and it was shown recently that inactivation of NGD factors was found to contribute to neurodegeneration. It remains to be established how these RNA degradation pathways specifically control these higher level functions—i.e., which specific RNAs involved in cellular differentiation and functions are targeted by RNA surveillance.

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