



Transcriptome surveillance in Aarhus, Denmark

# Prompt Control

Messenger RNAs are controlled for transcriptional mistakes while other RNA species control transcription itself. The “RNA world” is becoming increasingly complex these days, and ever more fascinating for people like Torben Heick Jensen (see photo below, 4th from right) and his group at Aarhus University.



**F**rom DNA to RNA to protein. As easy as this sounds it is, in fact, not. Aside from regulation of gene expression at the transcriptional level, including transcription factors and epigenetic events like DNA methylation and acetylation, which control the rate of gene transcription, there are also regulatory mechanisms following the transcription of DNA into mRNA.

This post-transcriptional regulation is the research focus of Torben Heick Jensen from the Department of Molecular Biology at Aarhus University, Denmark, who has been interested in this topic since his PhD, “I worked on the HIV-1 post-transcriptional regulator Rev and how it manages to shift the viral life cycle from one predominated by fully spliced viral RNA species to one where intron-containing RNAs suddenly enter the host cell cytoplasm, creating a complete shift in viral protein synthesis. This really caught my attention for gene regulation at the post-transcriptional level; an appetite which only grew stronger during a subsequent postdoctoral period with Michael Rosbash at Brandeis University, USA.”

## Health check

This appetite has recently led Jensen’s team to a couple of exciting discoveries, including novel, non-coding RNA transcripts with a possible role in transcriptional regulation and an updated model of nonsense mRNA degradation in human cells.

Transcriptome surveillance or quality control is an important mechanism for the

cell to make sure that only “healthy”, mature mRNAs reach the cytoplasmic ribosomes for translation. This is probably why degradative activities are always in the vicinity of mRNA productive steps. If processing fails, degradation takes over. “Survival of the fittest at the molecular level”, Jensen comments on this internal battle, which allows for the high accuracy of expression of genetic information.

Degradation of faulty mRNA can take place both in the nucleus, right after transcription, as well as in the cytoplasm when translation has already started. Amongst the cytoplasmic decay pathways are:

- ▶ the no-go decay (NGD) pathway, which degrades mRNAs after translation has halted at stable RNA structures;
- ▶ the non-stop decay (NSD) pathway, which degrades mRNAs lacking termination codons;
- ▶ and the nonsense-mediated decay (NMD) pathway, in which mRNAs with premature stop codons are eliminated. The NMD pathway hereby protects the cell from synthesising potentially harmful C-terminally truncated polypeptides.

It was previously found that in the fruit fly *Drosophila melanogaster* the NMD pathway is initiated by endonucleolytic cleavage near the premature termination codon (PTC). In mammalian cells, however, degradation was so far thought to merely occur exonucleolytically from the RNA termini. Jensen’s team, together with their collaborator Oliver Muhlemann from the Univer-

sity of Berne, wanted to know for sure. By applying RNA interference, they depleted human HeLa and HEK293 cells of 5’-3’ exonucleases as well as of components of the 3’-5’ exosome complex, which functions as 3’-5’ exonuclease, and thereby succeeded in showing that in mammalian cells the first step of NMD can also be endonucleolysis (*Nat. Struct. Mol. Biol.* 16(1): 49-55).

The group also identified the metazoan-specific NMD factor SMG6 as the responsible endonuclease. Therefore, in their revised model of human NMD, similar to *Drosophila*, SMG6 first cleaves the faulty mRNA endonucleolytically close to the ectopic termination codon. After that, the cleavage products are rapidly degraded exonucleolytically by the exosome complex or by alternative pathways.

## ‘Softening’ paradigms

Discovering that this RNA degradation pathway is apparently evolutionary conserved, at least since we shared common ancestors with the fruit fly, might raise the question of whether human cells are in any way “superior” compared to other eukaryotic cells when it comes to quality control of the transcriptome. Jensen, who is also the director of the Center of mRNP Biogenesis and Metabolism established by the Danish National Research Foundation in July 2005, explains, “I think, it is still an open question to which extent NMD pathways really vary in different organisms. New fields tend to rapidly create paradigms that are slow-

ly 'softened' as more data becomes available. Certainly, similar RNA degradative tools are available in all eukaryotes – even in prokaryotes.”

As if this wasn't already enough, Jensen and his group last year made another ground-breaking discovery: a new species of RNA. As he recalls, “Identifying PROMPTs (*PROM*oter *uP*stream *T*ranscripts) was a big surprise. They are produced upstream of most, if not all, active human promoters and constitute small (100-2000nt) unstable transcripts.”

At that time, it was already known that human cells could produce certain non-coding RNA species like short, promoter-associated RNAs. These are located about 0.5 kb on both sides of the transcription start sites (*Science* 316(5830):1484-8) and possibly play a role in RNA-directed modulation of gene transcription (*Proc. Natl. Acad. Sci. U S A.* 104(30): 12422-7).

Related transcripts were also found in a very different organism, yeast, “These cryptic unstable transcripts (CUTs)”, as Jensen explains, “had previously been described as being produced from intergenic regions of the *S. cerevisiae* genome.” They are degraded by the exosome complex immediately after their synthesis but, even though the precise functions of CUTs are yet to be determined, they nevertheless might exert a role in post-transcriptional regulation. In 2005, for example, Harvard geneticists were able to show that the regulation of an intergenic transcript interferes with adjacent promoters of coding regions and, in this way, controls gene expression in *S. cerevisiae*. (*Genes Dev.* 19(22): 2695-704)

### Opportunistic forces in action?

In an attempt to find hitherto undetected species of non-coding, short-lived RNAs in human cells, Jensen's team once again applied RNA interference technology to eliminate RNA degradation. The group knocked down a core component of the human exosome, hRrp40, in HeLa cells and subsequently analysed the RNA populations of the manipulated cells by high-density tiling microarrays. Overall, they observed a stabilisation of mRNAs. However, the surprise was that transcripts from small, non-genic regions, immediately upstream of active transcription start sites, also showed increased stabilisation. The PROMPTs were discovered (*Science*, 322(5909): 1851-54). Jensen explains, “Although PROMPTs and CUTs share some fundamental properties, there are some marked, and possibly important, differences. Finding related RNAs

in the much larger human transcriptome was not a shock, but also was not exactly anticipated.”

However, unearthing these non-coding RNA transcripts certainly leads to an even bigger question: what is their possible function? As with the CUTs in yeast, this question still remains to be answered in detail. “I think it's fair to say, that we still don't have a general answer as to whether PROMPTs are merely side-products of gene activity or more directly involved in the transcription process”, Jensen speculates. “However, there are many examples how the opportunistic force of evolution has turned former side-products into something useful.”

### Open chances for creativity

And so we've come back to evolution again. The “survival of the fittest” at the RNA level, conserved degradation mechanisms in multi-cellular organisms. There is so much we can learn from basic research into small creatures like *S. cerevisiae*, set out to reveal the fundamental mechanisms of how a cell manages to get all the right genes expressed at the right time or what goes wrong in genetic diseases like cancer. “It is very often the case that basic research turns out to be medically highly relevant,” Jensen reflects. “However, such insights can rarely be planned since great discoveries almost exclusively occur by chance. The important point is that creativity and free thought are given ample chances. If your research ideas are too preconceived you never discover anything original.”

Many of Jensen's and his colleagues' ideas have proven to be creative and it is very likely that, in the near future, even more exciting discoveries regarding post-transcriptional regulation will find their way from Aarhus University to the rest of the world. Jensen, at least, knows where they are heading, “We will continue to examine aspects of the synthesis and quality control of eukaryotic transcriptomes. We are interested in potentially undiscovered functions of new RNAs and/or the transcriptional mechanisms producing them. Another largely unanswered question is how cellular surveillance systems manage to discriminate between useful and wasteful transcription events. We are currently taking a stronger systems biology focus to try and tackle some of these questions more generally. However, rigorous analyses at the single gene or transcript level will never grow out of fashion and will continue to be absolutely essential for revealing mechanistic details.”

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