The great regulator

Basic research into the roles of RNA is steaming ahead, but its commercial exploitation has stalled

Philip Hunter

The discovery of RNA interference (RNAi) in 1998 sparked a revolution in RNA research (Fire *et al*, 1998). It also triggered a surge of investment from biotechnology companies and major pharmaceutical companies to develop new drugs and therapies that exploit this new mechanism of gene regulation. The commercial interest intensified in 2006 after Andrew Fire and Craig Mello received the Nobel Prize for their discovery of RNAi. Almost immediately, Merck invested US\$1.1 billion to acquire Sirna Therapeutics, which had several RNAi-based drugs in the pipeline or in clinical trials. This quickly persuaded other pharmaceutical companies to pile in, including Roche, Pfizer and Novartis. Since then, however, impatience with the rate of progress and the side effects and delivery problems of some RNAi-based therapeutics has caused Roche and Pfizer to divest their RNAi investments, and Novartis to reduce its commitment.

The temporary slowdown in commercial impetus is in contrast to the pace of basic research into RNAi and the molecules involved, which is barrelling ahead

The temporary slowdown in commercial impetus is in contrast to the pace of basic research into RNAi and the molecules involved, which is barrelling ahead. Technical advances in microscopy, deep sequencing and computation have led to a wave of discoveries that highlight the key role played by RNA in regulating gene expression and transcription, and that are creating renewed scope for RNA-based therapeutics.

New methods [...] have also revealed details of how RNA carries information from the nucleus to ribosomes for protein production

"I would say deep sequencing has been the most dramatic advance in RNA research technology over the last five years," commented Torben Jensen, who specializes in RNA quality in the Department of Molecular Biology at Aarhus University in Denmark. Deep sequencing uses various techniques to purify small RNAs and perform reverse transcription. This is followed by amplification to yield DNA samples that can be sequenced and analysed to identify, in principle, all small RNA molecules present in a cell at a given time.

It has led to major advances in understanding the central role that RNA molecules have, for instance during infection by bacteria or viruses. "What's really become evident over the last five years is that there are large networks of RNAs, many of which get induced during infection: small RNAs on the pathogen side [...] and non-coding RNAs on the host side," said Jörg Vogel from the University of Würzburg, Germany. These insights have the potential to lead to more-specific anti-bacterial drugs that could address the problem of antibiotic resistance. Surprisingly, however, that opportunity has yet to be grasped, Vogel explained. "Currently you can often identify the pathogens but can't tell yet what active state they are in. If we could exploit expression of small pathogen RNAs we could do this."

More fundamentally, deep sequencing has revealed that RNAs have a role

in gene regulation that is as important as that of protein transcription factors. "I think this is the biggest surprise for me, that networks of regulatory small RNAs in eukaryotes and also bacteria begin to rival transcription factors," said Vogel. "That's a game changer in terms of network function. It means RNAs become integral parts of gene expression networks." Yet, the topic needs more research to elucidate the way in which RNA and protein transcription factors interact, and the extent to which they complement each other.

Similarly, scientists are discovering more details about the role of small RNAs in quality control, in which they help to regulate messenger RNA (mRNA) levels by targeting certain transcripts for degradation. This is related to the recent finding that most of the genome is being transcribed most of the time in higher eukaryotes—albeit at a low level—rather than just the small part of it that contains protein-encoding genes. "Most of the genome now seems to be transcribed somewhere, not necessarily within a given cell type," said Jensen. "It seems then that a very important role for RNA quality control is to remove a lot of this RNA that is not useful."

...there are three mechanisms involving RNA that have already been exploited for drug development

The question is why so much non-coding RNA is produced at all if it has to be degraded immediately. The answer could be that it allows the cell to quickly generate new proteins when needed—the systems are on 'standby'. "I believe production of this unstable RNA is an interesting way of

producing new transcripts,” Jensen explained. “In yeast for example, if there is a shift in the sugar or carbon source it is growing on, you get massive changes in what RNAs are produced, [suggesting that] the transcriptome is capable of changing its profile quite rapidly by just having to alter the RNA, rather than having to turn on a protein factor.” Such environmental cues might also lead to epigenetic changes at the DNA level through chromatin modification, Jensen added, which might also be driven by RNA changes in response to shifts in the food source or environment.

New methods, such as fluorescence microscopy, have also revealed details of the way in which RNA carries information from the nucleus to ribosomes for protein production. One of the most significant discoveries is how translational silencing is combined with RNA transport to allow the cell to control exactly when and where genes are expressed. Michael Kiebler and colleagues at the Centre for Brain Research at the Medical

University of Vienna in Austria have gained new insights into RNA localization, a process that involves exporting RNAs from the nucleus in granules to the correct place for their expression (Mikl *et al.*, 2011). “Most RNAs are exported from the nucleus and translated at ribosomes in the cytoplasm,” Kiebler explained. “But the concept of forming RNA granules in the nucleus allows RNAs to be exported into the cytoplasm without immediately being seen by ribosomes [...]. Instead, upon remodelling, these RNA granules are targeted to the cytoskeleton, often to microtubules, in order to be transported to their final site of action.”

“Correcting aberrant splicing is important because a large percentage of diseases are caused that way, so there is a tremendous potential impact”

This process makes sense because mRNAs are smaller and easier to transport than the proteins they encode. RNA granules

therefore carry factors that determine when the RNA can be expressed. “This allows for fine tuning of gene expression locally in many ways,” Kiebler explained.

Although commercial exploitation of the role of RNA in transcriptional and epigenetic control is a long way off, there are three mechanisms involving RNA that have already been exploited for drug development. The first mechanism, small-interfering RNAs (siRNAs), involves small, double-stranded RNA molecules around 20 nucleotides in length that attach to their target mRNA through direct base-pair linking. Once attached, they cause the RNA-degradation machinery to cleave the mRNA at that site. The second mechanism, antisense microRNAs (miRNAs), involves similar RNA species, but instead of causing cleavage, miRNAs bind to the mRNA molecule to prevent it from being transcribed. The third mechanism with therapeutic potential is the correction of faulty splicing. This occurs when a normal gene is misread; if either an intron, a non-coding DNA

region within a gene sequence, is aberrantly transcribed; or part of an exon, containing the active coding sequence, is missed out by the RNA-splicing machinery. "Correcting aberrant splicing is important because a large percentage of diseases are caused that way, so there is a tremendous potential impact," commented Fritz Eckstein from the Max Planck Institute of Experimental Medicine in Göttingen, Germany.

Although Europe has islands of excellence [...] it does not have the same level of commitment as the USA...

In some cases, the aberrant splicing is caused by a mutation, as in Duchenne muscular dystrophy, which is the most common childhood genetic disease that affects 1 in 3,500 newborn boys. Duchenne muscular dystrophy causes progressive muscle weakness followed by heart and respiratory failure, and premature death. One of the most promising therapeutic approaches involves exon skipping to bypass deleterious mutations and restore expression of the muscle protein dystrophin. A small, synthetic RNA molecule makes the splicing machinery skip over the damaged part and the adjacent exon, in order to create a shorter mRNA that nonetheless yields active dystrophin. This method has been shown to be promising and safe in clinical trials, both in the Netherlands and the UK (Wood *et al*, 2010).

Some promising candidate drugs have also been developed on the basis of other well-understood RNA mechanisms. One example is a drug for treating the viral liver disease hepatitis C, based on the discovery that replication of the virus is stimulated by a particular miRNA, known as miR-122. "This particular microRNA is expressed in the liver and helps replication of the virus through a mechanism that is not yet well understood," Eckstein said. Treatment with an antisense oligonucleotide resulted in a dramatic decrease in expression of the viral RNA in liver cells (Jopling *et al*, 2005).

Yet, some of these emerging approaches have come up against difficulties during the past five years, which partly explains the retreat of some of the large pharmaceutical companies. One of the more difficult issues has been that some siRNA-based therapeutics achieve

their action by stimulating the immune system, rather than through specific RNAi. This was the case with a drug designed to treat 'wet' macular degeneration in adults—a disease that causes sight loss owing to the abnormal development of blood vessels. Drugs that were supposed to specifically knock down vascular endothelial growth factor, which stimulates angiogenesis in the eye, turned out to be activating toll-like receptor 3, which stimulates a general immune response against the unwanted blood vessels in the eye (Kleinman *et al*, 2008). "These siRNAs were the first to hit clinical trials, but are now out of the picture," noted John Rossi from the Department of Molecular and Cellular Biology at the Beckman Research Institute (Hope, CA, USA). "These findings should serve as a good lesson to the field of RNAi therapeutics that the use of naked, unmodified siRNAs should be avoided for most clinical applications, unless the triggering of TLRs is part of the therapeutic strategy."

After this prominent set-back, the siRNA field was fortunate that evidence soon came to light that siRNAs could be delivered to humans to treat solid cancers (Davis *et al*, 2010). This and other findings convinced Rossi and others that RNA-based therapeutics have a future, and that the recent set-backs are only temporary. "RNA drugs are here to stay," Rossi concluded.

However, unlocking their full potential and persuading pharmaceutical companies to renew their commitment requires stronger support for, and better organization of, fundamental research. This was the conclusion of a report by the European Science Foundation (Strasbourg, France), which proposes the creation of a virtual Europe-wide RNA institute, to connect research centres and provide a coherent framework for both fundamental and disease-focused research (ESF, 2010). The report also highlighted a greater commitment to basic research in the USA, where the RNA Therapeutics Institute (RTI) at the University of Massachusetts in Worcester has a similar brief, albeit focused purely on biomedical applications. "To the best of my knowledge, the RTI is the only academic centre that seeks to bring basic researchers, translational scientists, and clinicians together to think about RNA as a cause, diagnostic, or cure for human disease," said Phillip D. Zamore, co-director of the RTI.

Zamore urged Europe to move forward with its RNA virtual institute, suggesting that this would reflect a global shift in priority towards RNA research. However, the proposal of the ESF has yet to be taken up, according to Vogel, who co-chaired the ESF report. "At the moment nobody really knows how to proceed," Vogel said. "I definitely plan to return to this issue and pursue it this autumn." Although Europe has islands of excellence, according to Vogel, it does not have the same level of commitment as the USA, where momentum is still growing despite recent cuts in overall research funding.

In the end, the futures of RNA research and RNA therapeutics might be tightly intertwined. With a renewed focus on value for money from research, policy-makers might look to scientists to find ways to apply their knowledge in the clinic, even if big pharmaceutical companies remain cautious. Yet, as the important role that RNA has in gene regulation becomes clearer—and which diseases and disorders are caused by its mis-regulation—commercial exploitation for human health will certainly follow.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

REFERENCES

- Davis ME, Zuckerman JE, Choi CH, Seligson D, Tolcher A, Alabi CA, Yen Y, Heidel JD, Ribas A (2010) Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* **464**: 1067–1070
- ESF (2010) *RNA World: A New Frontier in Biomedical Research*. Strasbourg, France: European Science Foundation
- Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**: 806–811
- Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P (2005) Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. *Science* **309**: 1577–1581
- Kleinman ME *et al* (2008) Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. *Nature* **452**: 591–597
- Mikl M, Vendra G, Kiebler MA (2011) Independent localization of MAP2, CaMKII α and β -actin RNAs in low copy numbers. *EMBO Rep* [Epub 26 Aug] doi:10.1038/embor.2011.149
- Wood MJ, Gait MJ, Yin H (2010) RNA-targeted splice-correction therapy for neuromuscular disease. *Brain* **133**: 957–972

Philip Hunter is a freelance journalist in London, UK.

EMBO reports (2011) **12**, 894–896.
doi:10.1038/embor.2011.164