Technologies bring new era for RNA research

EUROCORES RNAQuality meeting held in Granada

A transformation in understanding of how genes and their expression are controlled by RNA molecules has taken place over the last decade with the help of technical advances in imaging, bioinformatics, and rapid sequencing. This has revealed a vast previously undiscovered population of mostly small RNA molecules with diverse roles way beyond the originally conceived function as a messenger (mRNA) carrying the genetic information encoded in DNA to the ribosome where proteins are made. Functions of small RNAs include protection of the mRNA, stabilisation of protein synthesis, regulation of genome structure, and resistance to viruses through silencing of their genes after transcription. Another important aspect lies in degradation of RNA molecules within the cell's cytoplasm, either because they are defective or because they have served their purpose for the time being.

Many of these functions, and the methods used to elucidate them, were discussed at a recent meeting held in Granada, Spain, during June 2008 and organised by the EUROCORES (European Collaborative Research) Programme RNAQuality of the European Science Foundation (ESF). Two principle areas of technical advances apparent at the conference involved structural analysis of molecules via x-ray crystallography or electron microscopy, and identification of RNAs within whole genome sequences.

One of the challenges for structural analysis lies in producing high quality crystals of protein or RNA molecules for x-ray crystallography , which relies on the ordered geometrical arrangement of multiple atoms within a crystal to produce coherent electron density images from scattered x-rays that enable the positions of the elements to be determined.

This problem was tackled through a combination of seeding techniques and crystallisation robots by Haiwei Song Associate Professor at the Institute of Molecular and Cell Biology in Singapore. Song used these techniques to derive the structure of the helicase core of the human version of the protein called Upf1, which plays an important role in decay of unwanted RNA molecules, or so called "nonsense RNA". Seeding is widely used to encourage crystal formation, but in the case of proteins is challenging partly because of the delicacy of the samples. Song used a micro-robotic technique that automates some of the steps involved in crystal production, while also ensuring that the crystals are good enough to yield accurate high structural data, in some cases enabling resolutions well below 3Å (angstrom units, or 0.1 nm) to be derived.

While electron microscopy does not usually match the resolution of X-ray crystallography, it is getting closer and has several other advantages, including the ability to obtain snapshots of different functional states, and produce images from small amounts of complexes in solution, according to Helen Saibil who runs a prominent cryo electron microscopy laboratory at Birkbeck College, London and who attended the EUROCORES RNAQuality conference. Saibil, who is also an investigator within the RNAQuality Programme, pointed out that X-ray crystallography and electron microscopy now complement each other very

well, with the former providing the atomic-level structure, while the latter is better at probing variations and functional details within large complexes comprising proteins and RNAs, such as the ribosome. “The most useful scenario is to have atomic structures of the components of a complex, or one state of a complex, by X-ray crystallography, or NMR spectroscopy (Nuclear Magnetic Resonance – another high resolution imaging technology), and then examine the range of functional states by cryo EM (electron microscopy), as is being done for the ribosome,” said Saibil.

Saibil also drew attention to the vital role of recent advances in computational and statistical analysis of images in the study of important functional elements that are mobile or vary between samples. “For many (if not most) samples of macromolecular machines, the sample contains a mixture of states, with slightly different conformations due to the presence or absence of ligands or other components,” said Saibil. “The problem is to distinguish structural variations from orientation variations - tricky, but becoming more feasible, with very large data sets and statistical analysis or probabilistic methods like maximum likelihood.” (Maximum likelihood is a statistical method that in this case can resolve structural variations between complexes in large, mixed data sets).

While x-ray diffraction can provide very high resolution, it cannot track the actual assembly and transport of RNA or associated protein molecules within the cell in real time. This requires some of the latest advanced cameras and lens systems for fluorescence microscopy, in which molecules of interest are tagged with fluorescent proteins that emit light and so allow a process to be followed in real time, at resolutions as high as 15 nm in some cases. This is about 30 times better than traditional light microscopy. At the RNAQuality conference, Michael Kiebler from the Medical University of Vienna’s Centre for Brain Research, presented work in which time-lapsed video fluorescence microscopy was used to follow the assembly of the ribonucleoprotein particles (RNPs) that transport RNA in living neurons. “Here we used Zeiss axiovert fluorescent microscopes equipped with very sensitive CCD cameras, such as the MicroMax or the CoolSnap HQ,” said Kiebler. The Kiebler laboratory has also generated RNA molecules in vitro and labeled them directly with fluorescent tags, then injecting them into mature hippocampus neurons. “Shortly upon injection, the assembly of the RNA into RNPs in the cell body and the subsequent transport along microtubules into dendrites is again followed by timelapse video microscopy in living neurons,” said Kiebler. Use of such advanced real time imaging is shedding light on the actual processes and mechanisms involving RNA.

Apart from imaging, the other important technical aspect of RNA research is the analysis of whole genome sequence data in the search for RNAs, rather than the physical structure. This field also requires advanced algorithms, in this case to locate short sequences that correspond say to small RNAs. These sequences can be identified because they are very similar to other sequences of RNAs that have already been identified.

One of the most important new techniques to obtain RNA sequence data is Deep Sequencing. This emerged from the widely used microarrays, which measure the expression of specified target genes. Deep sequencing goes further by measuring activity across the whole genome, including genes transcribed into RNAs that do not code for proteins and

instead have some regulatory function. Such advanced technologies have enabled the mystery of so-called junk DNA that does not code for proteins to be at least partially solved – much of this non-coding DNA produces small RNA molecules involved in regulation of genes. At one time it was thought all RNA coded for proteins, but it turns out that nearly all of it has other functions involved in regulating the genetic machinery rather than producing its protein output.

A recently developed type of microarray, known as a tiling array, is now being widely used. As the name hints, the technology involves placement of overlapping probes attached to the surface of the array in a tiling arrangement. The technology enables in depth analysis across the whole genome, and was used by several projects presented at the EUROCORES RNAQuality conference, including one led by Torben Heick Jensen, who used tiling arrays to uncover new small human RNAs normally targeted RNA exosome, a complex of proteins that plays a major role in the vital process of RNA degradation.

Rapid progress in all these technologies is currently being made and will give further impetus to the hot topic of RNA quality research. The 16 research groups from nine European countries collaborating in ESF's RNAQuality Programme aim at contributing to these developments, by using multidisciplinary approaches in diverse model systems and profitting from the synergies established in the programme.

Notes to editors:

The RNAQuality Programme comes under the ESF's EUROCORES (European Collaborative Research) scheme and will last 3 years. The aim of the EUROCORES Scheme is to enable researchers in different European countries to develop collaboration and scientific synergy in areas where European scale and scope are required to reach the critical mass necessary for top class science in a global context. The scheme provides a flexible framework which allows national basic research funding and performing organisations to join forces to support excellent European research in and across all scientific areas.
Until the end of 2008, scientific coordination and networking is funded through the EC FP6 Programme, under contract no. ERAS-CT-2003-980409. As of 2009, the National Funding Organisations will provide the funding for the scientific coordination and networking in addition to the research funding.

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For further information on RNAQuality, please go to www.esf.org/rnaquality
For further information on the EUROCORES Scheme, please go to www.esf.org/eurocores

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