Special Topic: Frontiers in RNA Research

Non-coding RNAs: a new RNA world

By Ling Wang

These days, non-coding RNAs (ncRNAs) including small RNAs and long ncRNAs (lncRNAs) are among the hottest topics in biology. Various types of ncRNAs and their functions have been discovered: from the well-understood tRNAs, rRNAs and miRNAs to the relatively less known piRNAs and lncRNAs. As more and more mysteries about ncRNAs are resolved, scientists look toward new methodologies and technologies to further explore the ncRNA wonderland. Haifan Lin and Yijun Qi, two renowned biologists based in the USA and China, respectively, recently spoke with NSR about their research and views on ncRNAs.

A ‘HOT TOPIC’ IN BIOLOGY

NSR: Why has ncRNA become a hot topic with an explosion in the number of research papers published?
Lin: The word ‘explosion’ indeed vividly describes this phenomenon. Over the past decade, the field of ncRNA research has rapidly expanded, thanks to the advent of the deep sequencing technology and completion of various genome projects. Deep sequencing has given us opportunities to effectively seize and characterize these elusive ncRNAs. When Nobel Prize winner Robert W. Holly first identified tRNA, he used 140 kg of commercial baker’s yeast, which yielded just 1 g of purified tRNA for sequence analysis of some 70 nucleotides. By comparison, today as little as 1 µL of solution is sufficient for deep sequencing billions of nucleotides. Furthermore, the completion of Human Genome Project and other genome projects provides us with maps and coordinate systems for gene research.
Qi: In the last decade, analyses using high-throughput technologies including tiling array and deep sequencing have led to a surprising finding that a large proportion of the genome sequences are transcribed but less than 2% of them are protein-coding. In another word, up to 98% of the DNA in our chromatin is non-coding. These sequences, previously known as ‘junk DNA’ or ‘dark matter’, now have emerged as a wonderland that gives birth to numerous small RNAs and lncRNAs, which are involved in a wide range of biological processes.

METHODOLOGIES: ALL ROADS LEAD TO ROME

NSR: Professor Lin, your work focusing on stem cells began with laser beams and mutant fruit flies and you are now exploring piRNA-mediated mechanisms in regulating stem cell behavior and development. Is this a typical approach to research ncRNAs?
Lin: Our research approach to study ncRNAs is to start with biological functions or a top-down approach. We started by trying to identify an important biological question. The important question to us was the mechanism of stem cell renewal. We then used genetic approach to identify key genes involved in this
process. Genetic approach is powerful because, generally speaking, the more obvious a phenotype you have observed, it means the more important a gene you’ve discovered.

Here is our story. To study stem cells, we first needed to identify them definitively. To this end, we transplanted the tip of an ovary from one fly to another and were able to regenerate ovaries even in male flies. This transplantation technique combined with laser ablation allowed us to identify stem cell in the ovarian germline and observe their division. We then wanted to know what controls their division. To address this question, we looked for genes involved in this process. Our hypothesis was that there must be genes expressed in stem cells or outside stem cells that are vital for stem cell division or differentiation, and that mutating any of these genes should lead to corresponding defects in stem cell division or differentiation. By this kind of reasoning, we found a number of genes important for stem cell self-renewal or differentiation. One of them was Piwi. Our search for Piwi-like genes in different organisms allowed us to discover the Argonaute/Piwi gene family, which was the first and still the only gene family known to be required for stem cell self-renewal in both animal and plant kingdoms. We then again used genetic approach to further identify other genes that interact with the Piwi gene in regulating stem cells. Among the eight interacting genes that we identified, four were later found to encode piRNAs.

In addition to the genetic approach, three other approaches are also important and commonly used in ncRNA research: biochemical, genomic and methodology development.

The biochemical approach aims to find specific molecules from cells or tissues and analyze their functions; this is in some sense a bottom-up approach. If you use this approach, you tend to identify molecules that are stable or abundant, but initially with unknown biological significance. However, this is a great approach to follow up genetic discoveries.

Genomic approach includes, but is not limited to searching the genome with computer assistance, and tries to locate these ncRNAs and obtain information on their possible regulatory function.

Method development approach is also very important. For example, improvement of the DNA sequencing technology has facilitated the discovery of many more ncRNAs.

These four approaches are deeply intertwined, and all have the same common goal: to address a biological question. Yijun is an excellent example of effectively combining these approaches in research, and this has allowed him to make important discoveries in the ncRNA field.

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—Haifan Lin

Qi: Using a right experimental system, asking an important question and/or developing a new technology are the key factors that lead to new discoveries. The finding of ncRNAs including piRNAs is indeed a perfect example of this.

Lin: And I must say that cooperation and communication are also essential in ncRNA research. For example, in the past years we benefited greatly from working closely together with structural biologists and other experts.

For another example, there was a milestone meeting in the early days of ncRNA research at Cold Spring Harbor called the Banbury Meeting, organized by Gregory Hannon and David Baulcomb, where a small number of biologists (myself included) shared their unpublished results, which promoted the subsequent rapid progress of the field.

NSR: What are differences between somatic ncRNAs and ncRNAs from stem cells?

Lin: There is no fundamental difference, and many researchers are working on what you called somatic ncRNAs. Since my interest is in stem cells and germline development, I focus on finding ncRNAs expressed in stem cells or the germline. Because these stem cells are multipotent and totipotent, respectively, ncRNAs in these cells might be more likely involved in developmentally important mechanisms.

NSR: ncRNA research is a multidisciplinary field, with scientists of various areas engaged. Is it still a wonderland full of excitement and frustrations?

Lin: Yes, it is. Scientists form a community, some with expertise in biology, some in chemistry and some in informatics and so on. Researchers make new discoveries from time to time, but there are still many unsolved problems. For example, when we first found piRNAs, we had no idea about their function. It could be just some junk RNA. We did not know about their function until we dug deeper. For another example, Yijun’s work on role for small ncRNAs in DNA repair is a fascinating discovery. We could not imagine such a role for RNA just a few years ago, but Yijun has done beautiful work to demonstrate this role.
Qi: Despite great efforts that have been made by many labs, I think we are still very far from a full understanding of ncRNAs. Our finding of the small RNAs involved in DNA damage repair (we call them diRNAs) was not expected by us either. We are excited by this surprising role for small RNAs and are working really hard to understand how they reach out for DNA repair. Given the unexpected complexity of ncRNAs, both in depth and breadth new strategies, methodologies and technologies will be needed to reveal what they do and how they do.

Lin: I totally agree that we have a similar dilemma. For a brilliant scientist in the ncRNA field, raising a good question may not be as difficult as solving them. I believe that there is always a reason for existence. There must be reasons why ncRNAs become prevalent during the long process of evolution.

RAPID PROGRESS IN CHINA

NSR: There are lots of talented researchers in China.
Lin: Correct, and they have done beautiful works! Scientists like Yijun who came back to China after impressive research experiences in the USA or Europe are working at the forefront in this area. They are energetic and creative, and not limited by their research condition compared with their elder peers in China. For example, last year I read an impressive Developmental Cell paper from Haoqin Liu’s lab in Shanghai, which demonstrates that a lincRNA maintains human embryonic stem cell self-renewal by working as a sponge to trap a miRNA that represses the translation of core pluripotency factors: Oct4, Nanog and Sox2. It, like Yijun’s work, also shows that Chinese scientists can achieve cutting-edge discoveries if given good support.

Qi: In China, funding for science and technology has been increasing dramatically over the last 10 or 20 years. I feel lucky and thankful that I have sufficient resource to do the science that I like since I started my own lab about eight years ago. The RNA research community in China is growing very fast. We have organized a national RNA club and meet twice a year to exchange information and discuss ideas. We are also hopeful that a big grant for RNA research will be started soon.

Lin: That is good! I am a member of a stem cell research strategic planning program in the Ministry of Science and Technology of China, and I hope that the money will be directed toward excellent researchers who will make breakthroughs in the area.

MYSTERIES TO BE ExpLORED

NSR: What do you think are the biggest challenges in the ncRNA field?
Lin: Biological function, molecular mechanisms and how to transfer basic research into clinical application still remain as challenging areas of ncRNA research.
Qi: Yes, and I would like to add that development of new strategies and approaches will be very much needed to solve these problems.

NSR: The public is more interested in the clinical application of ncRNA research. Is there some progress in this area?
Lin: Yes. There are companies developing microRNAs as medicines or as diagnosis tools. With further progress, I believe that ncRNAs will be an important component of the next-generation medicine.

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—Yijun Qi

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