SIGNAL TRANSDUCTION

Miniature magnetic force probes

Magnetoplasmonic nanoparticles can manipulate cell surface receptors with single-molecule precision to clarify the effects of force application and receptor clustering.

Mechanosensitive proteins have a variety of roles, ranging from the perception of inputs to developmental processes. To study the function of such proteins and the signaling pathways they participate in, one can apply mechanical force with tweezer-manipulated microbeads or with the tip of an atomic force microscope. However, "these microprobes are really big compared to the target proteins," says Young-wook Jun from the University of California, San Francisco. In collaboration with Zev Gartner's team at the University of California, San Francisco, Jun and his team set out to develop tools that are comparable in size to proteins.

The researchers followed up on their earlier work on monovalent quantum dots, which are fluorescent particles that can be targeted to single proteins. The nanoparticles in their latest study not only can be targeted to single proteins as well as imaged, but also can exert force on the target proteins. The particles have a size of about 50 nanometers and consist of a magnetic core and a plasmonic gold shell for manipulating and imaging the particles, respectively. The nanoparticles are coated with an oligonucleotide that is functionalized with a small molecule or protein that targets the nanoparticle to the mechanosensitive protein of interest. Because of its small size and monovalent nature, each nanoparticle attaches to a single target protein.

Jun and his colleagues manipulate the cell-attached nanoparticles with micromagnetic tweezers. Depending on the applied force and the distance of the tip from the cell, the researchers can cluster the nanoparticle-bound proteins or pull on single proteins. Jun explains that the beauty of his nanoparticle-based technology is that it makes it possible to distinguish spatial segregation of target proteins from mechanical activation. "That is not possible with the traditional microbead technology," says Jun. Owing to the larger size and multivalent nature of microbeads, many receptors are typically recruited, and therefore the mechanical activation

STRUCTURAL BIOLOGY

TOPOGRAPHICAL TRANSCRIPTOMES

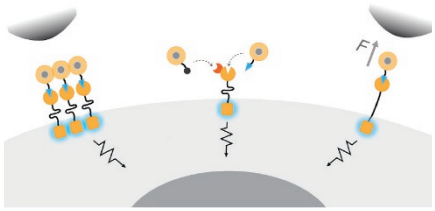
Three experimental methods generate global maps of *in vivo* RNA interactions.

Four decades ago, psoralen was shown to enter nucleic acid helices and cross-link opposing strands in the presence of UV light. Researchers began exploiting this property to map double-stranded regions in RNA, but with limited throughput. Three studies have now used psoralen to map RNA structure and interactions across the transcriptome.

Most high-throughput sequencing-based methods for exploring RNA structure work *in vitro* or focus on secondary structure; some can locate base-pairing regions but not the identities of pairing nucleotides. But proximity ligation, in which physically close RNA fragments are ligated to each other, has been used with a protein bait to detect long-range interactions. A group led by Jay Shendure at the University of Washington recently used *in vivo* RNA proximity ligation (RPL) to map base-pairing interactions transcriptome-wide without a protein bait.

RPL does not stabilize RNA base-pairing, however, and may miss weaker interactions. To address this issue, Stanford University researcher Howard Chang and his collaborators combined proximity ligation with cross-linking to devise PARIS (psoralen analysis of RNA interactions and structures), and Benjamin Blencowe and colleagues at the University of Toronto did the same with LIGR-seq (ligation of interacting RNA followed by high-throughput sequencing). Both methods apply a psoralen derivative and UV exposure for *in vivo* cross-linking, followed by gentle nuclease digestion to define duplex regions. RNA ligation, cross-link reversal and sequencing then reveal direct base-pairing between fragments. PARIS enriches for cross-linked duplexes by 2D electrophoresis, whereas LIGR-seq digests un-cross-linked RNA and normalizes against psoralen-free samples.

A third method, SPLASH (psoralen cross-linked, ligated and selected hybrids), from Niranjan Nagarajan, Yue Wan and colleagues at the A*STAR Genome Institute of Singapore, operates by the same principle but uses biotinylated psoralen for enrichment by streptavidin and uses chemical fragmentation rather than digestion.



Clustering (left), ligand activation (center) and mechanical activation (right) of Notch receptors. Adapted from Fig. 1 in Seo *et al.* (2016) with permission from Elsevier.

of the receptors cannot be separated from the effect of clustering these proteins.

Jun's team has applied their technology to clarify the roles of mechanical activation and oligomerization in Notch activation. Although the importance of mechanical activation has been recognized, it was unclear whether Notch oligomerization or ligand binding was necessary as well. The researchers discovered that mechanical activation alone was sufficient to initiate Notch proteolytic cleavage and downstream signaling processes. Furthermore, the team examined the differential effects of clustered E-cadherin or E-cadherin under mechanical stress and observed differences in the underlying actin cytoskeleton.

Jun thinks that his technology will be extremely useful for studying how mechanoreceptor signaling is regulated by spatial segregation and mechanical activation in a variety of contexts. In addition to Notch and E-cadherin signaling pathways and their roles in developmental processes, he is investigating the mechanism of mechanosensory ion channel signaling.

Jun is actively working on improving and expanding the technology. He explains that because of the small size of the nanoparticles, the magnetic field has to be approached very closely, which is difficult to control. In addition, the forces that can be applied through the nanoparticles are quite weak. By increasing the size of the magnetic core while decreasing the size of the shell, he aims to develop nanoparticles that are capable of generating greater forces.

Nina Vogt

RESEARCH PAPERS

Seo, D. *et al.* A mechanogenetic toolkit for interrogating cell signaling in space and time. *Cell* **165**, 1507–1518 (2016).

In human cell lines, the methods detected interactions with lower folding energies than shuffled sequences, were reproducible across replicates, and recapitulated many known structures and interactions, in particular between small nuclear RNAs involved in splicing. They exhibited good precision and sensitivity, capturing structures in low-abundance RNA. PARIS achieved near-base-pair resolution.

The work uncovered a host of new interactions in all RNA classes, a substantial fraction of which were intermolecular or separated by more than 200 nucleotides. LIGR-seq found unexpected interactions between small nucleolar RNAs and mRNAs, one of which was shown to regulate mRNA turnover. SPLASH found that mRNAs with end-to-end interactions tend to be translated efficiently whereas interactions within the 5′ region tend to inhibit translation. It also detected differences between human embryonic stem cells and cells differentiated using retinoic acid, highlighting the fact these approaches can uncover condition-specific and dynamic structures. In addition to human cell experiments, PARIS was carried out in mouse embryonic stem cells, and SPLASH was used in yeast; both studies found evidence of conserved RNA structures, and the PARIS authors used their data to guide evolutionary covariation analysis for refining structured regions.

The methods do have some bias: psoralen preferentially cross-links pyrimidines, and the efficiency of cross-linking and ligation is affected by duplex length, composition and accessibility. The greatest benefit will come from combining cross-linking with complementary approaches to understand RNA structure and cellular networks. With the aid of chemical probing data and phylogenetic conservation, for example, PARIS could identify inter-repeat duplexes in the difficult A-repeat structure of Xist RNA, critical for its role in X chromosome silencing.

Tal Nawy

RESEARCH PAPERS

Lu, Z. *et al.* RNA duplex map in living cells reveals higher-order transcriptome structure. *Cell* **165**, 1267–1279 (2016).

Aw, J.G.A. *et al.* In vivo mapping of eukaryotic RNA interactomes reveals principles of higher-order organization and regulation. *Mol. Cell* **62**, 603–617 (2016).

Sharma, E. *et al.* Global mapping of human RNA-RNA interactions. *Mol. Cell* **62**, 618–626 (2016).