Research Highlights

Crystal structure of a eukaryotic group II intron lariat

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When film editors throw away bad takes and stitch together their final cut, they unknowingly mimic one of the most fundamental processes of life, splicing. Within our cells illegible sections of genes, known as introns, are removed by splicing to produce coherent messages used as directions for making proteins. These excised introns have a unique branched circular shape known as lariats, reminiscent of a cowboy's lasso.

Researchers in Assistant Professor Navtej Toor's lab in the Department of Chemistry and Biochemistry at UC San Diego, in collaboration with NE-CAT staff, have observed at atomic resolution how these ubiquitous lariat molecules are formed. Their research article was published in the journal *Nature* on October 9, 2014.

"It's all about the knot," said lead author Aaron Robart, a postdoc in the Toor lab (Fig. 7). "How the knot in the intron RNA is tied and how it's handled by the ribozyme is what splicing is really all about." In our cells introns are removed by one of the most complex molecular machines ever discovered: the spliceosome. To understand the atomic details of gene splicing the Toor lab looked to an unlikely source, the brown algae *Pylaiella littoralis*, which harbors highly active group II introns. Group II introns are the molecular ancestors of our splicing machinery but perform the same reaction using only one catalytic RNA molecule; providing a tractable model system to tackle the complexity of splicing.

This structure of the lariat also provides exciting new details of how metal ions fuel the splicing machine and provide specificity. "When it comes to splicing, accuracy is everything," said Jessica Peters (Fig. 7), coauthor and graduate student in the Toor lab. "It's almost hard to imagine but our cells perform this reaction constantly and if it's off even a little bit the results are disastrous." In humans the way a gene is pieced together by splicing allows one gene to make many different products and mistakes have severe penalties in the form of disease.

The new findings also provide a glimpse into

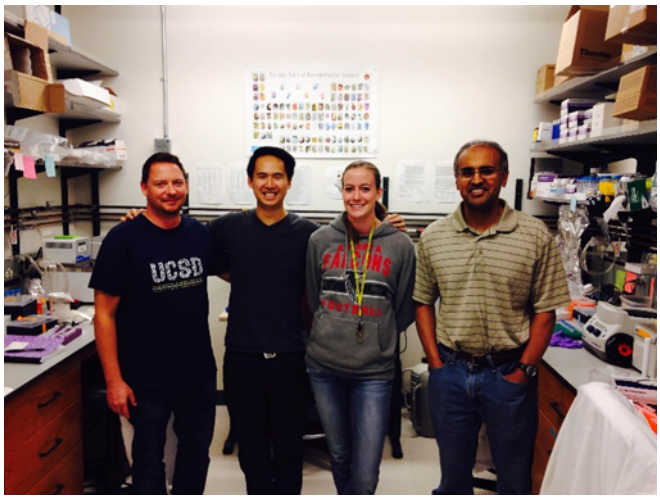

Fig. 7 The members of the Navtej Toor lab who participated in this research. From left to right: Aaron Robart, Russel Chan, Jessica Peters and Navtej Toor.

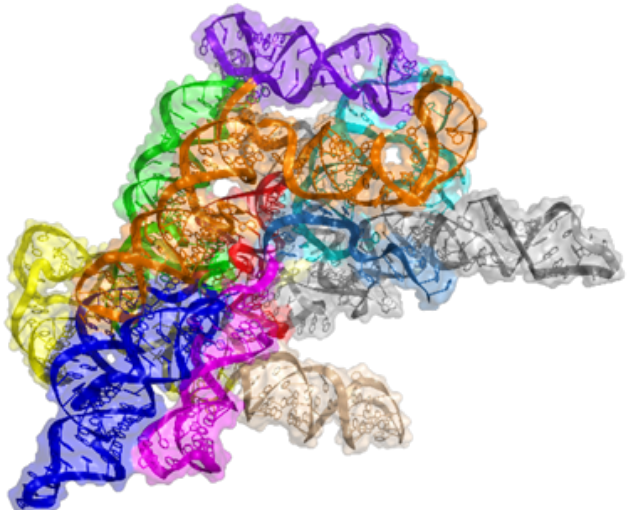

Fig. 8 Crystal structure of the group II intron lariat. Domain 6 is shown in purple and contains the bulged adenosine responsible for lariat formation.

our past. "What excites me is the role lariats have played in shaping our DNA landscape," said Russell Chan (Fig. 7), coauthor and senior graduate student in the Toor lab. "The unusual nature of the lariat bond is what allowed introns to lasso DNA and colonize our genomes." This intron invasion has indeed been prolific with at least half of our DNA being comprised of these non-coding elements.

The group II intron lariat structure revealed a host of new interactions between the intron RNA domains that explained roles for conserved secondary structure elements in structural stability and lariat formation (Fig. 8). Domain III was found to form a large external brace stabilizing a large segment of the structure through two long distance interactions: μ-μ′ and the newly discovered τ-τ′ tertiary interactions. Furthermore domain II, long thought to be unnecessary for splicing activity, was revealed to serve as a central organizational node by coordinating a confluence of four tetraloop-receptor interactions. Of these interactions the newly discovered π-π′ tertiary contact was particularly exciting due to its position directly adjacent to the bulged adenosine that forms the lariat bond. This new interaction plays a pivotal role in directing how the knot within the lariat RNA rope is handled in the splicing catalytic mechanism. The π-π′ interaction is predicted to pull the first splicing step lariat product out of the active site, simultaneously clearing the active site and allowing the second step reactants to enter. The structure also expanded the number of highly coordinated Mg^{2+} metal ions that fuel the splicing machine and provide specificity to the reaction. Two new metals were shown to position the 5' splice site by binding around the GUGYG sequence universally conserved in splice site recognition from group II to mammalian introns.

Looking to the future, the Toor lab aims to expand upon this snap-shot of splicing by obtaining high resolution structures along each step of the splicing path. This will ultimately provide a complete molecular understanding of the intricate details required for proper processing of our genes by splicing.

Staff Activities

Presentations

Frank Murphy, "Synchrotron Beamlines - It's Not Uphill Both Ways Anymore", Ribosome Alumni Meeting during LMB Alumni Symposium, Cambridge University, Cambridge, England, July 10-12, 2014.

David Neau, "Recent Developments at NE-CAT, a Macromolecular Crystallography Synchrotron Facility", Indo-US International Conference/Workshop on Recent Advances in Structural Biology and Drug Discovery, Indian Institute of Technology, Roorkee, India, October 9-11, 2014.

Surajit Banerjee, "NE-CAT: Crystallography Beamlines for Challenging Structural Biology Research" Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, Tennessee, August 1, 2014.

Posters

N. Sukumar. "A comparative analysis on X-ray structure of cobalamin binding proteins", 23rd International Union of Crystallography (IuCr) and General Assembly, Montreal, Quebec, Canada, August 5–12, 2014.

I. Kourinov, M. Capel, S. Banerjee, F. Murphy, D. Neau, K. Perry, K. Rajashankar, J. Schuermann, N. Sukumar, S. Ealick, "NE-CAT Crystallography Beamlines for Challenging Structural Biology Research", 23rd International Union of Crystallography (IuCr) and General Assembly, Montreal, Quebec, Canada, August 5–12, 2014.