施一公院士 Science 同期发表剪接体研究的姐妹文章

Yigong Shi published sister papers about spliceosome in Science

【Science 系列】说到 Science 上发表的中国科学家的文章，本季度最瞩目的就是清华大学的施一公院士课题组。清华大学的施一公院士课题组再度在剪接体研究中取得重大突破，2 篇姊妹研究论文同期发表在《科学》（Science）杂志。

施一公院士

施一公研究组于《科学》（Science）杂志就剪接体的结构与机理研究发表两篇长文（Research Article），报道了酿酒酵母（Saccharomyces cerevisiae）剪接体激活和剪接反应催化过程中两个重要状态的剪接体复合物近原子分辨率的三维结构，阐明了剪接体的激活和催化机制，从而进一步揭示了前体信使 RNA 剪接反应的分子机理。

在最新发表的两篇《科学》论文中，施一公研究组进一步探索并优化了蛋白提纯方案，捕获了性质良好的酿酒酵母剪接体分别处于激活状态（activated spliceosome，又称为 Bact complex）和第一步催化反应后（catalytic step I spliceosome，又称为 C complex）的优质样品，并利用单颗粒冷冻电镜技术和高效的数据分类方法，重构出了总体分辨率分别为 3.5 和 3.4 埃的两个高分辨率冷冻电镜结构，并搭建了原子模型。这两个复合物近原子分辨率三维结构的解析，首次完整地展示了第一步转酯反应前后 pre-mRNA 和起催化作用的 snRNA 的反应状态，以及剪接体内部蛋白组分的组装情况。尤为值得一提的是，催化核心区域的分辨率达到了 2.8 至 3.0 埃，清晰的展示出剪接反应中心的结构信息，为解释剪接体对 pre-mRNA splicing 的催化机制提供了迄今最为清晰的关键证据。

如上两个结构与该研究组之前报道的 ILS 剪接体及 2016 年 1 月报道的 3.8 埃的酿酒酵母 tri-snRNP 结构的对比更为深刻的揭示了剪接体在 pre-mRNA 剪接反应过程中作为核酶催化完成两步转酯反应的本质，是 RNA 剪接研究领域的又一突破性进展。

在题为“Structure of a yeast catalytic step I spliceosome at 3.4 Å resolution”的 Science 文章中，研究人员报告称采用冷冻电子显微镜获得了平均分辨率为 3.4 埃的酿酒酵母催化第一阶段剪接体（复合物 C）的原子结构。研究揭示的结构特征描述出了第一阶段催化反应后剪接体的形成，预测了完成第二阶段酯转移反应所需的结构改变。

在题为“Structure of a yeast catalytically activated spliceosome at 3.5 Å resolution”的 Science 文章中，研究人员称采用冷冻电子显微镜(cryo-EM)确定了来自酿酒酵母的催化激活剪接体（复合物 Bact）的原子结构，平均分辨率达到 3.52 埃。最终定义的模型包含 U2、U5 snRNP，U6 snRNA、NTC、NTC related (NTR)和一条包含 71 个核苷酸前体 mRNA(Pre-mRNA)分子，38 个蛋白总共 13,505 个氨基酸，结合分子量近 1.6 兆道尔顿。这一结构与早先确定的那些结构勾勒出了前体 mRNA 剪接反应的一个分子框架。

Structure of a yeast catalytic step I spliceosome at 3.4 Å resolution

第一步催化反应后的酵母剪接体 3.4 埃的结构

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Abstract

Each cycle of pre–messenger RNA splicing, carried out by the spliceosome, comprises two sequential transesterification reactions, which result in the removal of an intron and the joining of two exons. Here we report an atomic structure of a catalytic step I spliceosome (known as the C complex) from Saccharomyces cerevisiae, as determined by cryo–electron microscopy at an average resolution of 3.4 angstroms. In the structure, the 2′-OH of the invariant adenine nucleotide in the branch point sequence (BPS) is covalently joined to the phosphate at the 5′ end of the 5′ splice site (5′SS), forming an intron lariat. The freed 5′ exon remains anchored to loop I of U5 small nuclear RNA (snRNA), and the 5′SS and BPS of the intron form duplexes with conserved U6 and U2 snRNA sequences, respectively. Specific placement of these RNA elements at the catalytic cavity of Prp8 is stabilized by 15 protein components, including Snu114 and the splicing factors Cwc21, Cwc22, Cwc25, and Yju2. These features, representing the conformation of the spliceosome after the first-step reaction, predict structural changes that are needed for the execution of the second-step transesterification reaction.

Structure of a yeast activated spliceosome at 3.5 Å resolution

酵母剪接体激活状态 3.5 埃的结构

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Abstract

Pre–messenger RNA (pre-mRNA) splicing is carried out by the spliceosome, which undergoes an intricate assembly and activation process. Here, we report an atomic structure of an activated spliceosome (known as the B^{act} complex) from Saccharomyces cerevisiae, determined by cryo–electron microscopy at an average resolution of 3.52 angstroms. The final refined model contains U2 and U5 small nuclear ribonucleoprotein particles (snRNPs), U6 small nuclear RNA (snRNA), nineteen complex (NTC), NTC-related (NTR) protein, and a 71-nucleotide pre-mRNA molecule, which amount to 13,505 amino acids from 38 proteins and a combined molecular mass of about 1.6 megadaltons. The 5′ exon is anchored by loop I of U5 snRNA, whereas the 5′ splice site (5′SS) and the branch-point sequence (BPS) of the intron are specifically recognized by U6 and U2 snRNA, respectively. Except for coordination of the catalytic metal ions, the RNA elements at the catalytic cavity of Prp8 are mostly primed for catalysis. The catalytic latency is maintained by the SF3b complex, which encircles the BPS, and the splicing factors Cwc24 and Prp11, which shield the 5′ exon–5′SS junction. This structure, together with those determined earlier, outlines a molecular framework for the pre-mRNA splicing reaction.