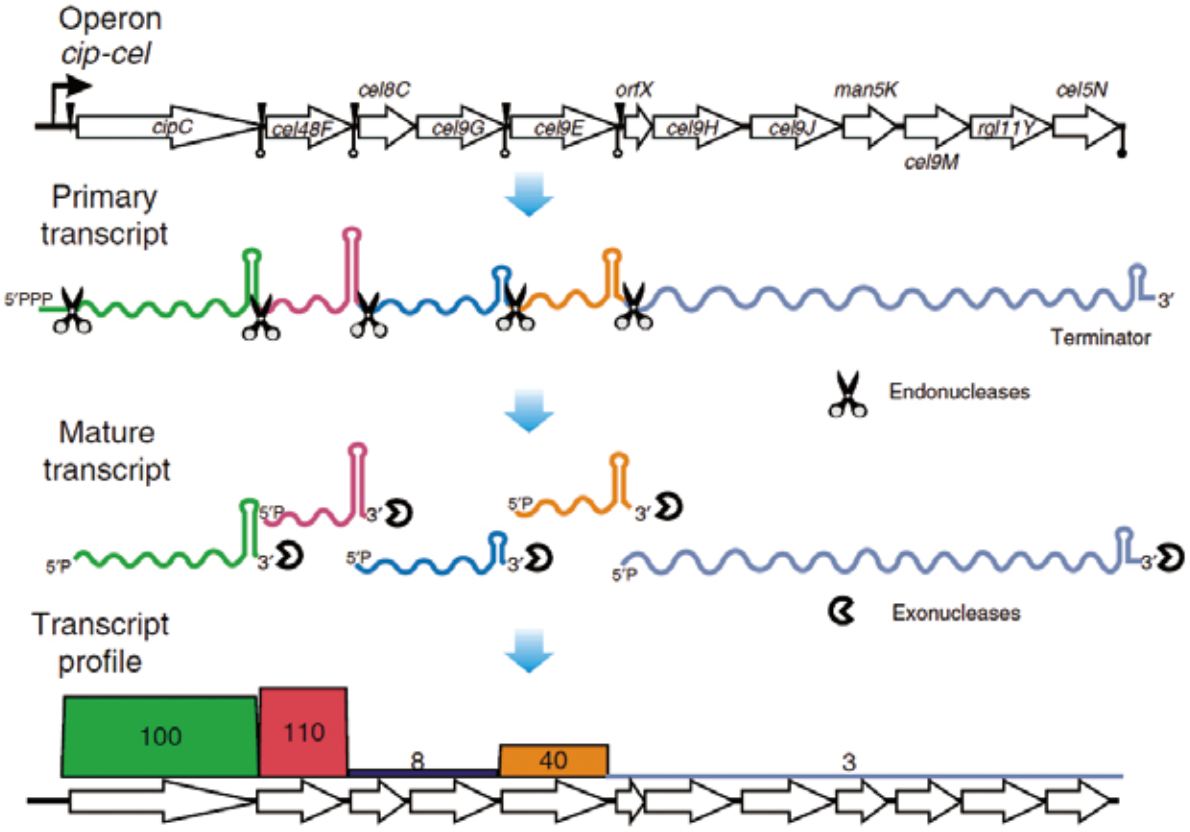
Research Reveals Regulatory Mechanism of Cellulosome Stoichiometry

Efficient biological degradation of cellulosic biomass has been recognized as one major bottleneck in production of cellulosic liquid fuels or biogas, and as the one key step in the carbon cycle of biosphere. In a new work published online in *Nature Communications* on 24 April, 2015, a research team from the Single-Cell Center of the Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences discovered a new mechanism in microbial degradation of lignocellulose, by reporting that cellulosome stoichiometry is regulated by selective RNA processing and stabilization (SRPS).

Cellulosome is the most efficient machinery for cellulose degradation found in nature, and is a high-molecular-mass protein complex assembled from extracellular enzymes. To achieve the proper function, it is crucial to maintain appropriate stoichiometry among these cellulosomal subunits. However, how do cells encode, recognize and control the information?

By mapping the genome-wide transcriptional start sites (TSs) and post-transcriptional processed sites (PSs) of transcripts via a differential mRNA-sequencing approach



A model for regulation of the stoichiometry of cellulosomal components in vivo. The *cip-cel* operon is transcribed by its sole promoter and the primary transcript is cleaved into several secondary transcripts by endonucleases as defined by iPSs. However, stability of these secondary transcripts against exonuclease degradation varied due to their distinct terminal structure. The resulted distinction in transcript level among the genes result in the observation of a "complex" type operon and eventually lead to the proper composition and ratio of cellulosome subunits in *C. cellulolyticum*.

(dRNA-Seq), Dr. XU Chenggang, graduate student HUANG Ranran and their colleagues from the Functional Genomics Group of the Single-Cell Center revealed that in the cellulolytic model organism *Clostridium cellulolyticum*, SRPS mechanism precisely regulates cellulosome stoichiometry at the post-transcriptional level.

The team found that the *cip-cel* cluster which encodes 12 cellulsomal subunits is transcribed as a single transcriptional unit, namely an "operon". However, these genes exhibit highly skewed transcript abundance, with a ratio of 100:110:9:8:38:5:4:2:3:2:3:5. This ratio of transcript abundance is correlated with the relative abundance at the protein level. The team revealed that at least five PSs located in the intergenic regions of the *cip-cel* operon are specifically recognized by endonucleases, resulting in cleavage of the primary transcript into at least six secondary transcripts. Stability of these secondary transcripts varied widely due to their distinct terminal stem-loop structures, which quantitatively determine the observed stoichiometry. Intriguingly, orthologous stem-loops were found in the intergenic regions of *cip-cel*-like operons from other Clostridia. Therefore, the PSs and the stem-loops precisely regulate structure and abundance of the subunit-encoding transcripts processed from a primary polycistronic RNA, quantitatively specifying a cellulosome "recipe", and drive evolution of bacterial cellulose degradation.

This simple and ingenious mechanism that controls stoichiometry of protein complex not only ensures simultaneous precise control of ingredients of cellulosome "recipe" following a certain ratio, but also allows reliable inheritance of the "recipe". Moreover, this discovery introduces a new approach to construct "Super Cellulosome" and "Super Cellulolytic Bacteria" and might enable applications in design and engineering of synthetic protein-machineries.

This work represents a collaborative effort from the Functional Genomics Group led by Dr. XU Jian and the Metabolomics Group led by CUI Qiu. It was funded by the Ministry of Science and Technology of China and the National Natural Science Foundation of China.