Splicing factor SRSF10 is known to function as a sequence-specific splicing activator that is capable of regulating alternative splicing both in vitro and in vivo. We recently used an RNA-seq approach coupled with bioinformatics analysis to identify the extensive splicing network regulated by SRSF10 in chicken cells. We found that SRSF10 promoted both exon inclusion and exclusion. Functionally, many of the SRSF10-verified alternative exons are linked to pathways of response to external stimuli. Here we describe in detail the experimental design, bioinformatics analysis and GO/pathway enrichment analysis of SRSF10-regulated genes to correspond with our data in the Gene Expression Omnibus with accession number GSE53354. Our data thus provide a resource for studying regulation of alternative splicing in vivo that underlines biological functions of splicing regulatory proteins in cells.

Key Laboratory of Food Safety Research, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

© 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).
SRSF10 both activates and represses exon inclusion

There are five major modes of alternative splicing (AS) events described in metazoan organisms, including cassette exon, alternative 3′ splice site, alternative 5′ splice site, mutually exclusive exon and retained intron (Fig. 2A). With a p value cutoff of < 0.05, we were able to identify 167 events belonging to the five major modes of AS from a total number of 10,652 splicing events that changed significantly between the WT and the KO samples by ASD. Specifically, the majority of the affected splicing events (130) belonged to the cassette exon and retained intron (Fig. 2A). With a p value cutoff of < 0.05, we were able to identify 167 events belonging to the five major modes of AS from a total number of 10,652 splicing events that changed significantly between the WT and the KO samples by ASD. Specifically, the majority of the affected splicing events (130) belonged to the cassette exon
category. Strikingly, analytical results by ASD indicated that SRSF10 could both activate and repress exon inclusion (Fig. 2B). RT-PCR validation of SRSF10-affected splicing events further demonstrated this prediction. In addition, the selection of alternative 3′ and 5′ splice sites was similarly affected by SRSF10. Taken together, these results provide strong evidence that SRSF10 can promote both exon inclusion and exclusion in vivo.

**Function enrichment analysis of SRSF10-affected genes**

To understand how loss of SRSF10 affects cellular biological processes, we next performed gene ontology (GO) and pathway analysis of SRSF10-affected genes by using ClueGO [6]. Function enrichment analysis indicated that these affected spliced genes are involved in multiple processes, including histone methylation, response to external stimulus, glucose metabolic processes and mitochondrion distribution (Fig. 3). These results illustrated that SRSF10 might play an important role in a wide range of biological processes by controlling its downstream splicing targets.

**Discussion**

We present here a unique dataset of RNA-seq results in which we globally analyzed SRSF10-regulated AS events based on deep sequencing of the chicken transcriptome. We found that SRSF10 is involved in all of the common modes of AS, with cassette exons being the most frequent targets for SRSF10 regulation. We also found that SRSF10 could both activate and repress exon inclusion in vivo. Finally, SRSF10-affected splicing genes were involved in multiple processes, indicating a role of SRSF10 in these processes. This dataset is of high quality and will facilitate future study of SRSF10 function in vivo and will also improve understanding of AS regulation in vivo.

**Acknowledgements**

We thank Dr. Manley (Columbia University) for his generous gifts of SRSF10 KO cells and anti-SRSF10 antibodies. This work was supported by grants from the Ministry of Science and Technology of China [2012CB524900]; the National Natural Science Foundation [31370786 and 31170753] and the One Hundred Talents Program of the Chinese Academy of Sciences.

**References**

[1] Y. Feng, M.T. Valley, J. Lazar, A.L. Yang, R.T. Bronson, S. Firestein, W.A. Coetzee, J.L. Manley, SRp38 regulates alternative splicing and is required for Ca(2+) handling in the embryonic heart. Dev. Cell 16 (2009) 528–538.

[2] Y. Feng, M. Chen, J.L. Manley, Phosphorylation switches the general splicing repressor SRp38 to a sequence-specific activator. Nat. Struct. Mol. Biol. 15 (2008) 1040–1048.

[3] C. Trapnell, L. Pachter, S.L. Salzberg, TopHat: discovering splice junctions with RNA-seq. Bioinformatics 25 (2009) 1105–1111.
C. Trapnell, B.A. Williams, G. Pertea, A. Mortazavi, G. Kwan, M.J. van Baren, S.L. Salzberg, B.J. Wold, L. Pachter, Transcript assembly and quantification by RNA-seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. 28 (2010) 511–515.

X. Zhou, W. Wu, H. Li, Y. Cheng, N. Wei, J. Zong, X. Feng, Z. Xie, D. Chen, J.L. Manley, et al., Transcriptome analysis of alternative splicing events regulated by SRSF10 reveals position-dependent splicing modulation. Nucleic Acids Res. (2014), http://dx.doi.org/10.1093/nar/gkt1387.

G. Bindea, B. Mlecnik, H. Hackl, P. Charoentong, M. Tosolini, A. Kirilovsky, W.H. Fridman, F. Pages, Z. Trajanoski, J. Galon, ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics 25 (2009) 1091–1093.