Plant Circular RNAs (circRNAs): Transcriptional regulation beyond miRNAs in plants

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Current plant functional genomics is converging on two aspects to provide sustainable solutions for feeding the growing population demands: 1) Engineering crops for sustainable food security, where recently identified *CRISPR/Cas* is playing a detrimental role (Nekrasov et al. 2013; Belhaj et al. 2015) and 2) identifying regulators of the post-transcriptional regulation, which can be functionally engineered. Canonical splicing has been widely seen and associated with functional protein diversity in plants (Filichkin et al. 2015) (Fig.1). Concurrent patterns of exonic and intronic splicing has revealed several new isoforms, whose aberrant canonical expression has been widely resulted in ecotypic specific responses to environmental stresses (Filichkin et al. 2015). Recent advances in the high-throughput sequencing has revealed a new potential class of endogenous RNAs, circularRNAs (circRNAs) (Jess and Sharpless 2014) (Fig.1), as a result of exon scrambling, back-end (backend, non-canonical splicing). Like other endogenous RNA regulators, such as microRNA (miRNA), variants of miRNAs (isomiRs) (Sablok et al. 2015), circRNAs are endogenous RNAs, which lack 5' or 3' ends, are resistant to exonuclease-mediated degradation and have been demonstrated to act as post-transcriptional regulators by canonical binding to miRNAs, thus have been classified as miRNA sponges (Hansen TB et al. 2013, Memczak S et al. 2013).

In humans, since the first report of the genomic based evidence of the circRNAs (Salzman et al. 2012; Salzman et al. 2013), abundance of non-canonical splicing originated circRNAs have been observed with as many as 100,000 circRNAs recently reported as per the large scale analysis of the ENCODE (Gao et al. 2015), which indicates that this class of regulatory RNAs are the next level of transcriptional regulators besides microRNAs. In plants, however the relative lack of such abundance of circRNAs has been seen except for previous little evidence of these regulatory RNAs across the tree of life (Wang et al. 2014). Recently, Ye et al. (2015) used rRNA depleted RNA-Seq (RibominusSeq) and by anchor aligning the reads to model plant *O. sativa* and *A. thaliana* revealed 12037 and 6012 circRNAs in *O. sativa* and *A. thaliana* respectively with over 700 orthologus exonic circRNAs in *O. sativa* and *A. thaliana*. Large scale identified circRNAs were from coding regions and revealed limited repetitive and reverse complementary sequences in intronic sequences flanking exonic circRNAs in plants as compared to animals (Ye et al. 2015).

Despite the observance that repetitive elements have been established as a regulator of circRNAs in animals (Wiluz 2015; Ling-Ling and Li 2015), plant circRNAs revealed relatively less association to repetitive elements and longer flanking introns as compared to those reported in animals. A corollary study in *O. sativa* by Lu et al. (2015) identified 2354 circRNAs among, which are 1356 exonic circRNAs with only 92 circRNAs were found to be enriched for miniature inverted-repeat transposable elements (MITEs) and supports Ye et al. (2015) observations of the relatively less association of the plant circRNAs to the repetitive regions. In plants, it might be proposed that alternative biogenetic pathways for circRNAs biogenesis in plants as compared to animals (Barrett et al. 2015; Chuan and Shan 2015), given the observance that relatively few circRNAs were linked to inverted repeats. (Ye et al. 2015, Lu et al. 2015)

A key question now stands in plants: does this endogenous class of regulatory RNAs originated through backsplicing share the same regulatory mechanism as alternative splicing? In humans, Wang et al. (2015) highlights the role of the cis-elements and general splicing factors in regulating the back-end splicing, however, the regulatory nature of these regulators is different as compared to the canonical splicing. Functional abundance, expression and translational efficiency of these regulatory RNAs is still a challenging area with recent reports using a single exon minigene containing split GFP (Green Fluorescent Protein) construct provides evidences for the translatable circRNAs (Wang et al. 2015). Analogous to their translatable nature, divergence in the expression patterns of the circRNAs can been in animals (Salzman et al. 2013). However, co-expression has not been widely observed in animals, which is in contrary to recently observed significant positive correlation between the exonic circRNAs and their parent genes in plants (Ye et al. 2015). The fate of functional relevance of the recently discovered exonic and intronic circRNAs has been tightly linked to the tissue specificity (Liu et al. 2015), which supports the previous observance in animals (Memczak et al. 2013; Salzman et al. 2013) and functional regulatory evidences of these circRNAs in plants have been seen in environmental stress such as phosphate imbalance (Ye et al. 2015).

circRNAs lessons gleaned from the so far available plant circRNAs doesn't imply the potential appositeness of circRNAs as microRNA sponges in plants as compared to animals (Hansen et al. 2013). To support, Lu et al. (2015) further validated the miRNA target mimics by overexpressing circRNA Os08circ16564 in a transgenic line, whose canonical miRNAs pertaining to family 172 and 810 targets important genes in rice spikelet and floral organogenesis. However, expression analysis between the empty-vector control plants and Os08circ16564-transgenic plants revealed no significant deviations but over expression of the circRNAs reduced the expression level of the parental gene (Lu et al. 2015). Despite the contrasting target mimics and relative difference in the observed biogenetic pathways, circRNAs converge on the tissue specific expression in both plants and animals and shed light on the new level of post-transcriptional gene regulation. Although the circRNAs reports in plant are relatively small as compared to animals where large scale portals such as CircNet (Liu et al. 2015) and Circ2Traits (Ghosal et al. 2013) has already been established to reveal the regulatory role of these circRNAs in disease trait association and circRNA-miRNA-gene regulatory networks (Liu et al. 2015), large scale detection and quantification of these circRNAs in complex polyploids such as *Triticum aestivum* (AABBDD), *Brassica rapa*, *Vitis vinifera* and other allo- and aneuploid species, where alternative splicing has largely contributed to the complex genome and ecotypic diversity can could accelerate the role of the discovery of these new functional regulatory endogenous RNAs and corresponding parental gene expression in a quest for finding the sustainable functional genomics assisted solution to feed the world.

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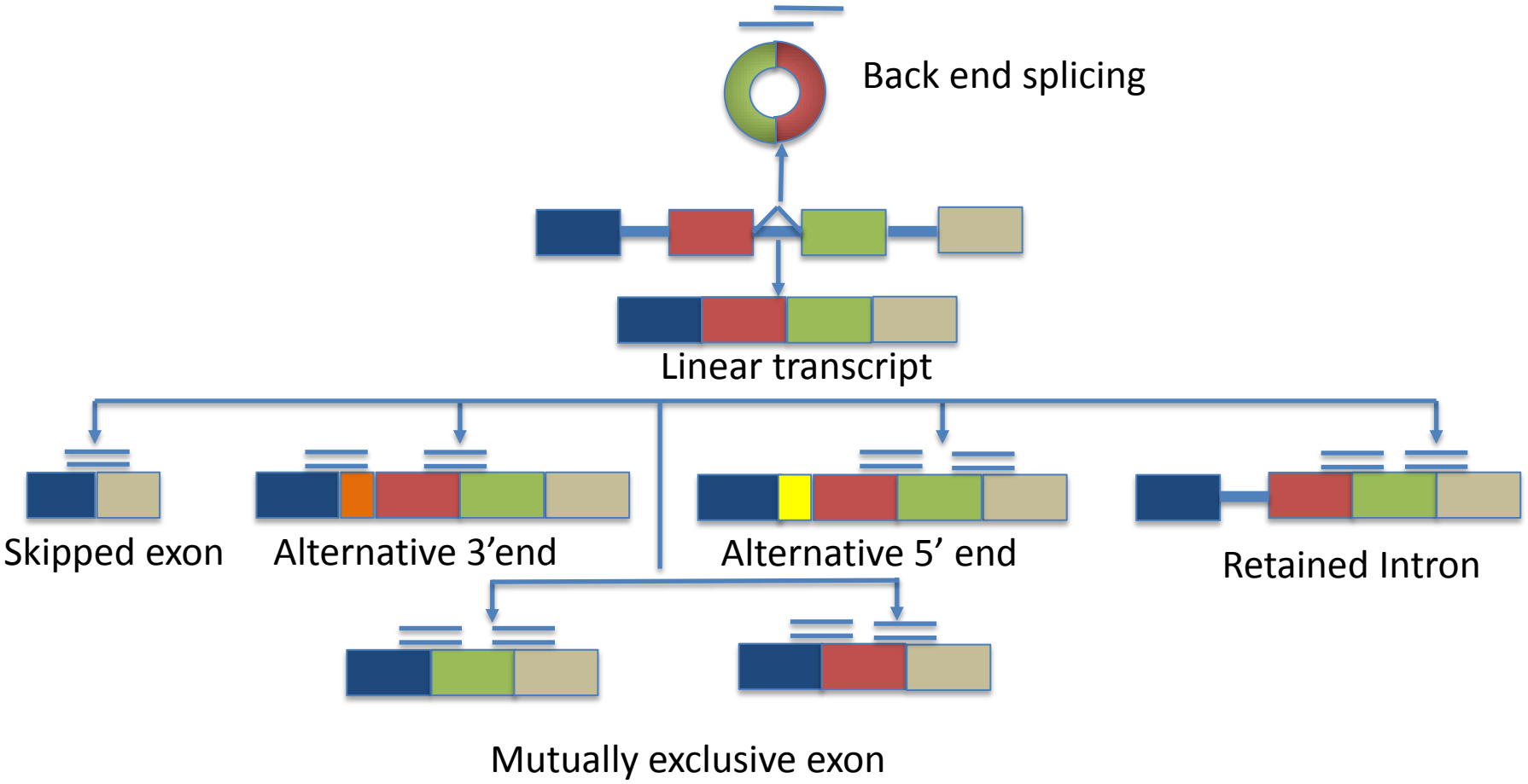
Figure legends:

Fig.1 Regulation of cellular proteome diversity through alternative and back-end splicing phenomenon



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