One gene many proteins: Mapping Plant Alternative Splicing

Ranjan Swarup[a], Martin Crespi[b] and Malcolm J Bennett[a]

[a]*Centre for Plant Integrative Biology, School of Biosciences, University of Nottingham, LE12 3RD, UK*

[b]IPS2, Institute of Plant Sciences Paris-Saclay (CNRS-INRA-University of Paris-Saclay, Bat. 630, 91405, Orsay, France

Corresponding Author: Malcolm J. Bennett, School of Biosciences, Sutton Bonington Campus, University of Nottingham, Loughborough, LE12 5RD, UK.

Phone +44 115 951 3255; E-mail: malcolm.bennett@nottingham.ac.uk

pre-mRNA alternative splicing (AS) generates protein variants from a single gene that can create novel regulatory opportunities. In this issue of Developmental Cell, Li et al. (2016) present a high-resolution expression map of AS events in Arabidopsis root tissues, giving insight into cell-type and stage specific AS mechanisms in plants.

The majority of eukaryotic genes are comprised of exons and introns. Their transcribed pre-mRNAs undergo RNA splicing, where introns are excised and exons are joined together to form mature messenger RNA sequences. Many RNA molecules undergo alternative splicing (AS) that generates two or more mRNAs from the same pre-mRNA, through the use of alternative acceptor and donor splicing sites that demark intron/exon boundaries (Figure 1A). This regulated removal of selected parts of mRNAs results in increased transcriptome and proteome diversity. AS has been implicated in many biological processes, including several diseases in mammals (Faial 2015) and regulation of abiotic stress responses in plants (Filichkin et al 2015). AS can also produce protein variants that exhibit differences in their stability, enzymatic activity, localisation, post translational modifications and protein-protein interactions

Recent estimates suggest that 95% of intron containing genes in humans (Pan et al 2008) and >60% of plant genes undergo alternative splicing (Marquez et al 2012). However, in most cases the functional significance of different spliced variants is not clear. Creating a catalogue of alternatively spliced transcripts and determining their functions will be crucial resource to address the role of AS in generating transcriptome and proteome diversity and assessing its impact on growth and development.

A new Resource study, Li et al. (2016), from Philip Benfey's group in this issue of *Developmental Cell*, presents a profile of alternatively spliced transcripts from multiple root tissues and developmental stages in the model plant *Arabidopsis thaliana*. Its roots represent an ideal system to perform such a study since cell type sorting methods are well established (Birbaum et al, 2005). In addition, root cells undergo division, expansion and maturation in spatially distinct meristem, elongation and

differentiation zones, respectively (see Figure 1B for schematic). The authors exploited the technical and anatomical advantages of using *Arabidopsis* roots in combination with next generation sequencing techniques to create a high resolution AS map of *Arabidopsis* root cell types to reveal new insights and examples of AS mechanisms in plants.

Several different types of AS have been described in the literature resulting from the use of alternative acceptor and donor sites (Carvalho et al 2012; see schematic in Figure 1A). Whilst exon skipping is widespread in metazoan systems (Kim et al 2007), this appears relatively rare in Arabidopsis (<5%). Instead, Li et al. (2016) observed that intron retention is the major type of AS in root tissues (>41%), in agreement with previous findings (Filichkin et al, 2010), followed by alternative acceptor (<26%) and alternative donor (>12%) splicing variants. The authors classified intron retention events as either type I (where the major isoform excises the intron) or type II (where the major isoform retains the intron). Interestingly, type II events are more likely to retain functional reading frames and are less likely to have in frame stop codons than type 1 events (<72% versus 13% respectively). Other recent work recently described these type II events as 'exitrons' (Marquez et al. 2015), which are more conserved than type I isoforms in plant crucifer species (88% versus 26%), highlighting their functional importance. Li et al. (2016) observed that type II events were much more enriched in specific cell types than type I, thereby predicting alternative functionalities of these proteins in different root cell types.

AS provides the opportunity to generate novel protein variants that can have functional specialization. For example, only one splice variant of the IBR5 MAPK phosphatase can function during auxin root growth responses (Jayaweera et al. 2014). To delve into the potential utility of their dataset, Li et al. (2016) examined the AS of a b-ZIP1 transcription factor gene called *ABSCISIC ACID RESPONSIVE ELEMENT* (*ABRE*) *BINDING PROTEIN 2* (*AREB2*). The *AREB2* transcript exists in different isoforms, including a major and common minor form termed isoform1 and isoform3, respectively. These isoforms are generated through their use of alternative splice acceptor sites in exon 4, resulting in a single amino acid sequence difference. Isoform 3 is normally highly expressed in the root differentiation (but not meristem or elongation) zone (Figure 1B). To test a potential role of AS in root cell differentiation, either isoform 1 or 3 were ectopically expressed in transgenic roots lacking *AREB2* and 2 closely related genes. Only lines expressing isoform 3 resulted in premature differentiation of root division and elongation zone cells when treated with the hormone signal abscisic acid. Further studies revealed that isoform 3 disrupts the ABA-dependent nuclear targeting of isoform 1. This example elegantly illustrates how AS can create new opportunities for regulation by modulating protein composition. The new dataset promises to be a rich treasure trove for researchers to mine and identify many other examples of AS regulation in plants.

Unlike animal systems where cell types generally express one specific alternatively spliced mRNA, Li et al. (2016) observed plant root cell types typically contain one major plus multiple minor isoforms. However, a pair wise comparison of AS events between different root cell types versus developmental

zones (i.e. cell division, elongation and differentiation zones) revealed >3 fold greater number of isoforms in the latter datasets. This observation suggests that AS regulation is more important for cell maturation rather than cell specification (as illustrated by the example of *AREB2* above). This begs the question, which mechanism is likely to drive AS in this cellular context? Splicing regulator Ser/Arg rich (SR) proteins have been linked to AS regulation. Consistent with this, Li et al. (2016) observed that transcripts of SR proteins are more differentially expressed between developmental zones than between cell types. However, it remains to be shown whether SR protein-driven AS events can regulate tissue differentiation, rather than cell specification in *Arabidopsis* roots.

Can these mRNA AS events relate to abundance of their protein isoforms? To address this important point, Li et al (2016) performed quantitative proteomics on samples isolated from 3 distinct root developmental zones (relating to cell division, elongation and maturation; Figure 1A). The authors detected a total of <17,000 peptides, including >300 specific for individual AS isoforms. Intriguingly, they observed a poor correlation between low abundance transcripts with moderate to high protein levels, but found a higher correlation between major isoforms and protein abundance, particularly between fold changes in transcript and protein abundance across the 3 root developmental zones profiled. Hence, the regulation of major isoform abundance appears to play an important role controlling levels of their respective proteins in the root. Future analysis of translatome regulation of AS variants will assess mechanisms that control root protein levels.

In summary, next generation sequencing (NGS) techniques are uncovering new insights about the regulation of pre-mRNA processing in plants. When combined with tissue-specific RNA isolation techniques as done by Li et al. *(*2016), NGS-based RNAseq datasets provide a rich resource to uncover novel AS regulatory mechanisms that impact cell specification and maturation.

(1001 words)

Figure 1. Alternative splicing (AS) events occurring in *Arabidopsis* root tissues and zones.
(A) Schematic representations of splicing scenarios reported by Li et al (*this issue*) including (top) 'normal' pre-mRNA splicing out of introns (lines) and stitching together of exons (boxes) versus AS events involving 'exon skipping', 'Intron retention', use of 'alternative acceptor' or 'alternative donor' splicing variants. (B) Schematic representation of *Arabidopsis* root tissues (see colour code inset) plus meristem and elongation zones where cell division and expansion takes place, respectively.

References (10)

Birnbaum K, Shasha DE, Wang JY, Jung JW, Lambert GM, Galbraith DW and Benfey PN (2003) *Science,* **302,** 1956-1960.

Carvalho RF, Feijao CV and Duque P (2012) *Protoplasma*, **250**, 639-650.

Jayaweera T, Siriwardana C, Dharmasiri S, Quint M, Gray WM, et al. (2014) *PLoS ONE* **9(8):** e102301

Faial T (2015) *Nat Genet*, **47**, 105.

Filichkin SA, Priest HD, Givan SA, Shen R, Bryant DW, Fox SE, Wong WK and Mockler, TC (2010) *Genome Res,* **20,** 45-58.

Filichkin SA, Cumbie JS, Dharmawardhana P, Jaiswal P, Chang JH, Palusa SG, Reddy AS, Megraw M, Mockler TC (2015) *Mol. Plant* **8**, 207–227.

Kim E, Magen A, Ast G and Aviv R (2007). *Nucleic Acid Res*, **35**, 125-131.

Li S, Yamada M, Han X, Ohler U, and Benfey PN (2016) *Dev Cell, this issue.*

Marquez Y, Brown JW, Simpson C, Barta A, Kalyna M (2012) *Genome Res,* **22**, 1184-1195.

Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ (2008). *Nat Genet*, **40**, 1413-1415

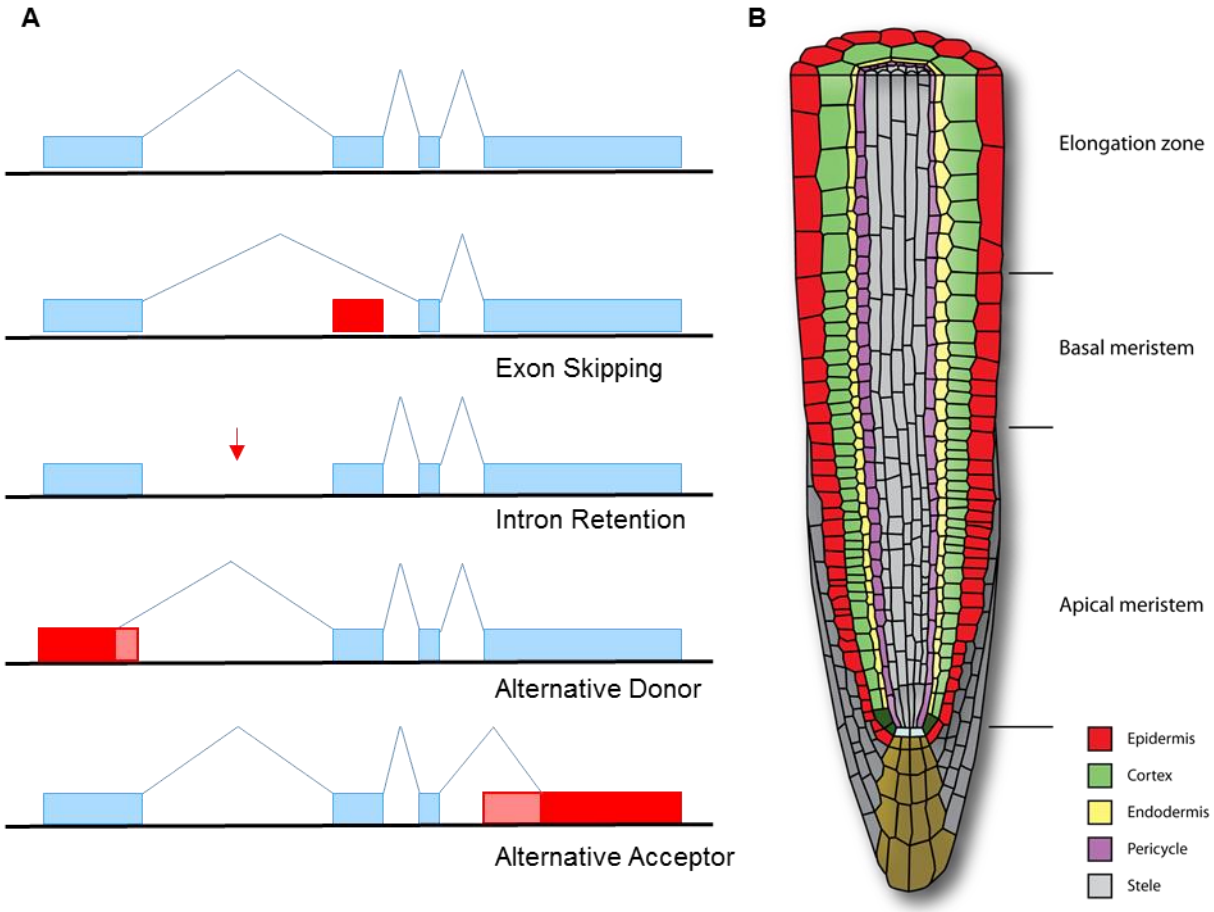

Figure 1. Alternative splicing (AS) events occurring in *Arabidopsis* root tissues and zones.
(A) Schematic representations of splicing scenarios reported by Li et al (*this issue*) including (top) 'normal' pre-mRNA splicing out of introns (lines) and stitching together of exons (boxes) versus AS events involving 'exon skipping', 'Intron retention', use of 'alternative acceptor' or 'alternative donor' splicing variants. Arrow indicates retailed intron. (B) Schematic representation of *Arabidopsis* root tissues (see colour code inset) plus meristem and elongation zones where cell division and expansion takes place, respectively.