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Alternative splicing in plants: directing traffic at the crossroads of adaptation and environmental stress

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In recent years, high-throughput sequencing-based analysis of plant transcriptomes has suggested that up to ~60% of plant gene loci encode alternatively spliced mature transcripts. These studies have also revealed that alternative splicing in plants can be regulated by cell type, developmental stage, the environment, and the circadian clock. Alternative splicing is coupled to RNA surveillance and processing mechanisms, including nonsense mediated decay. Recently, non-protein-coding transcripts have also been shown to undergo alternative splicing. These discoveries collectively describe a robust system of post-transcriptional regulatory feedback loops which influence RNA abundance. In this review, we summarize recent studies describing the specific roles alternative splicing and RNA surveillance play in plant adaptation to environmental stresses and the regulation of the circadian clock.

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Introduction

Alternative splice site selection during eukaryotic precursor-mRNA (pre-mRNA) processing results in the production of multiple mature mRNA isoforms from a single gene locus, known as alternative splicing (AS). AS expands proteomic diversity and regulates gene expression at the post-transcriptional level. Splice site selection has been shown to be regulated by cell type, developmental stage, and cellular stress. High-throughput Sequencing-based estimates of alternatively spliced transcripts in *Arabidopsis thaliana* range from 42% [1•] to 61% [2]. It is likely that many more genes will be shown to undergo AS as transcriptomes of plants grown under stress are evaluated and as computational tools used for NGS-based predictions of splice isoforms are improved [3,4,5•,6].

Alternatively spliced mRNAs in *Arabidopsis* can accumulate at substantial levels [1•,5•,7,8,9•]. AS frequently generates nonsense mRNA carrying in-frame premature termination codons (PTCs) [1•,2,5•,8,9•,10]. PTC-harboring ('PTC+') mRNAs are, in many cases, rapidly degraded by the cellular nonsense-mediated mRNA decay (NMD) machinery. This type of AS is often referred to as 'unproductive alternative splicing' [11•,12]. Some PTC+ mRNA escape NMD and produce truncated proteins which may be missing key functional domains. In plants, evidence suggests that stable NMD-insensitive PTC+ mRNAs play important roles in transcriptome adaptation to developmental demands and/or plant responses to environmental stresses [5•,9•,13]. In this review, we summarize recent findings describing mechanisms of AS, coupling of AS to the RNA surveillance machinery, and the specific roles of AS and NMD in plant adaptation to the environmental stress and in regulation of the circadian clock.

Pre-mRNA splicing machinery

Eukaryotic pre-mRNA splicing is mediated by a large ribonucleoprotein (RNP) complex known as the spliceosome. Both constitutive and AS of pre-mRNA is catalyzed by the spliceosome. This large RNP complex is comprised of five small nuclear RNAs, small nuclear ribonucleoproteins (snRNPs), and hundreds of spliceosomal proteins [14–16]. There are strong similarities in exon–intron structure and a significant conservation of splice site consensus signals within pre-mRNAs between plants and animals [17].

Members of the serine/arginine-rich (SR) protein family mediate spliceosomal pre-mRNA binding specificity. The SR proteins have a modular structure consisting of one or two RNA recognition motifs (RRMs) and an arginine/serine-rich (RS) domain [5•,18–20]. The RRM domains are involved in pre-mRNA splice site selection, whereas RS domains likely mediate protein–protein interactions and act as splicing activators [21]. Some SR proteins act as splicing repressors (e.g., dephosphorylated mammalian

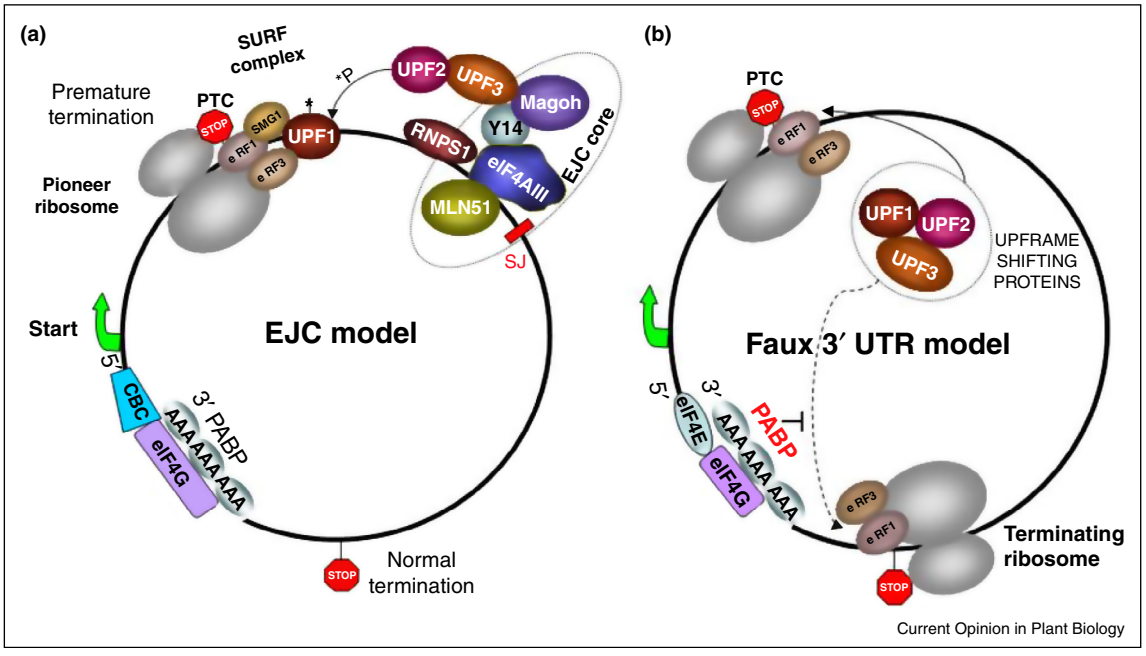
SRp38 inhibits splicing during heat shock), whereas other SR proteins antagonize the inhibitory activity of the heterogeneous nuclear RNPs (hnRNPs) to activate splicing [22].

Coupling of alternative pre-mRNA splicing to NMD

Alternatively spliced mRNAs harboring in-frame PTCs can be recognized by the RNA surveillance machinery as aberrant; these transcripts are targeted by the nonsense-mediated decay (NMD) pathway and rapidly degraded. Key current NMD models are illustrated in Figure 1. Recognition of an aberrant mRNA by NMD is determined by NMD-eliciting transcript features, such as PTC location relative to the initiation codon, length of the 3′ untranslated region (3′ UTR), and/or presence of short overlapping open reading frames in the 5′ UTR [23•]. A particularly potent NMD response can be triggered by short PTC-harboring exons (present in certain mammalian splicing factors and termed poison cassette exons [12]). In *Arabidopsis*, *HSFA2*, *RVE2* [24•], and *SR30* [1•] transcripts provide examples of such poison cassette exons.

Compatible models of on-demand switches toward unproductive AS via intron retention (IR) proposed for the fern *Marsilea vestita* [25,26••] and *Arabidopsis* [24•] are likely to be broadly relevant for other eukaryotic systems. Studies of masked mRNA storage during development of *M. vestita* microspores favor the hypothesis that IR mRNA intermediates escape cytoplasmic NMD via sequestration in the nucleus; these intermediates are spliced and released depending on cellular demands. Boothby and Wolniak [25] demonstrated that unspliced and partially spliced pre-mRNAs can be stored in nuclear speckles in

Figure 1



Current NMD models. **(a)** The exon junction complex (EJC) model was proposed for mammalian cells and relies on the position of the PTC relative to the downstream exon/exon junction and associated EJC. The EJC proteins are deposited onto spliced mRNA during pre-mRNA splicing and remain associated with spliced mRNAs during their transport into the cytoplasm. The EJC proteins are removed in the cytoplasm during the pioneer round of ribosome scanning, resulting in an NMD-recalcitrant mRNA. Upon premature translation termination, the downstream EJC recruits UPF factors. The subsequent formation of the EJC/UPF complex determines whether a stop codon is interpreted as being PTC. UPF2 and UPF3 recruit UPF1 to the EJC and cooperatively stimulate both the ATPase and RNA helicase activities of UPF1. NMD is triggered by interaction of the phosphorylated UPF1 protein with the ribosomal release factors (eRF1 and 3) during the pioneer round of translation. **(b)** The Faux (false) 3′ UTR model [55] presumes that NMD is regulated by interactions of a terminating ribosome with the 3′ UTR binding protein(s). Normal translation termination occurs only when a terminating ribosome is in close proximity to the 3′ UTR that allows interaction between eRF3 and the poly(A) binding protein complex (PABPC) associated with the 3′ poly(A) tract. Premature termination occurs when translation terminates distal from the 3′ UTR and the terminating ribosome cannot interact with PABPC but instead recruits NMD UPF factors. Plant NMD incorporates some features of both models. However, in contrast to mammalian cells where mRNAs transcribed from intronless genes can escape NMD, plant single exon transcripts harboring a PTC can be detected and degraded by NMD machinery. The cap-binding protein complex (CBC), an important component of the existing models, is not essential for NMD in plants [56]. The PTC position [57], 3′ UTR length [58] and the presence of the short upstream ORFs (uORFs) [59] appear to be important NMD-eliciting transcript features [23•]. The EJC proteins are conserved across many eukaryotes, however, direct involvement of plant EJC orthologs in plant NMD remains poorly studied. *Arabidopsis* eIF4A-III (an EJC 'anchor' on mammalian mRNAs) and ALY/Ref orthologs co-localize to the nucleolus together with Mago, Y14, and RNPS1/SR45 [60,61]. This finding suggests that the EJC-mRNA complex is at least assembled in plant cell nucleus.