Alternative splicing and plant stress

Craig G. Simpson, John Fuller, Jim McNicol, Diane Davidson & John W.S. Brown

Alternative splicing (AS) modulates gene expression during development and in response to external stimuli. When a gene is transcribed, introns are removed by splicing at the splice sites bordering the intron. Around a third of plant genes show alternative selection of splice sites leading to the production of more than one mRNA from the same gene by alternative splicing (Fig. 1A). The different AS transcripts can give rise to proteins with altered function or activity which affect the cell's characteristics. Therefore AS effectively expands the information content of the genome through increasing protein diversity allowing the plant to rapidly respond to immediate changes in conditions and providing one basis for selection for adaptation during evolution.

The importance of AS is well illustrated in humans where mutations in splice sites or regulatory splicing factors affect AS with profound effects contributing to diseases such as muscular dystrophy, Parkinson's disease and cancer. In plants, the involvement of AS in regulating plant phenotypes and responses is reflected in the many examples of AS in genes involved in development, disease and stress responses as well as in transcription and RNA-interacting factors. For example, resistance to *Tobacco mosaic virus* and the "sticky" rice phenotype are both due to alternative splicing.

AS is also a key area where gene expression is regulated. It is controlled by the composition of multiple protein factors (such as SR or hnRNP proteins) and their interaction with sequences on gene transcripts. The relative levels or activity of these proteins change in different cells or conditions through transcription, protein modification or localisation, so the alternative splicing pattern of sets of genes is altered and different proteins with different activities are produced (Fig. 1B). For example, different hormones such as ABA and stress conditions can activate phosphorylation signalling pathways which will alter the phosphorylation state of splicing regulators, affecting AS of downstream target genes. Thus, the characteristics of different cell types, cells at different stages of development or in response to external stimuli are dependent on the cellular code of

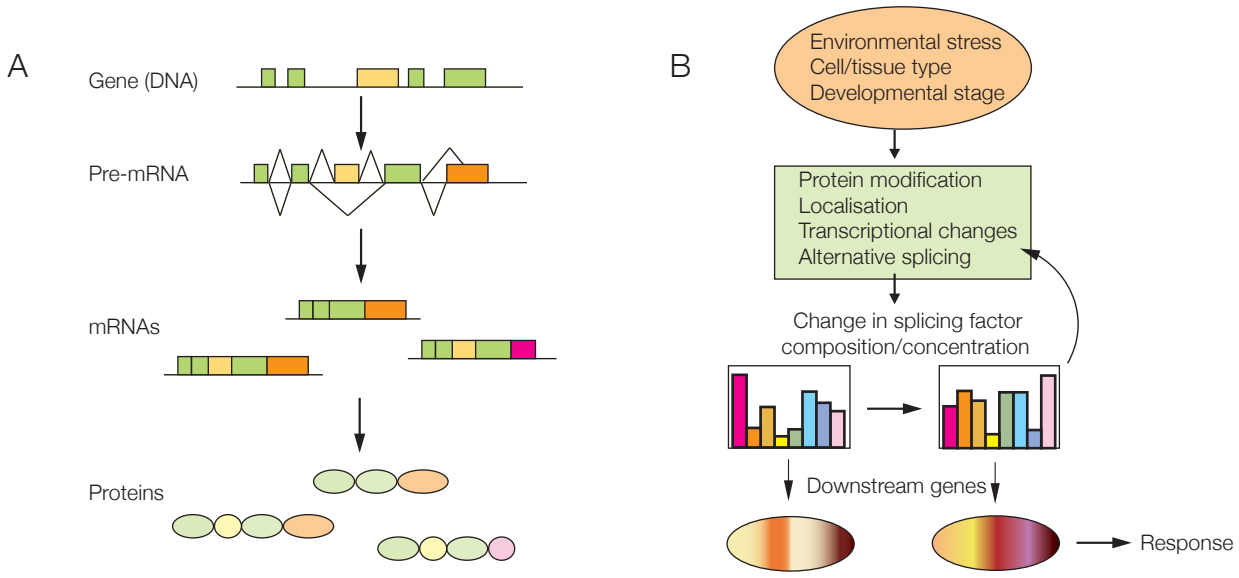
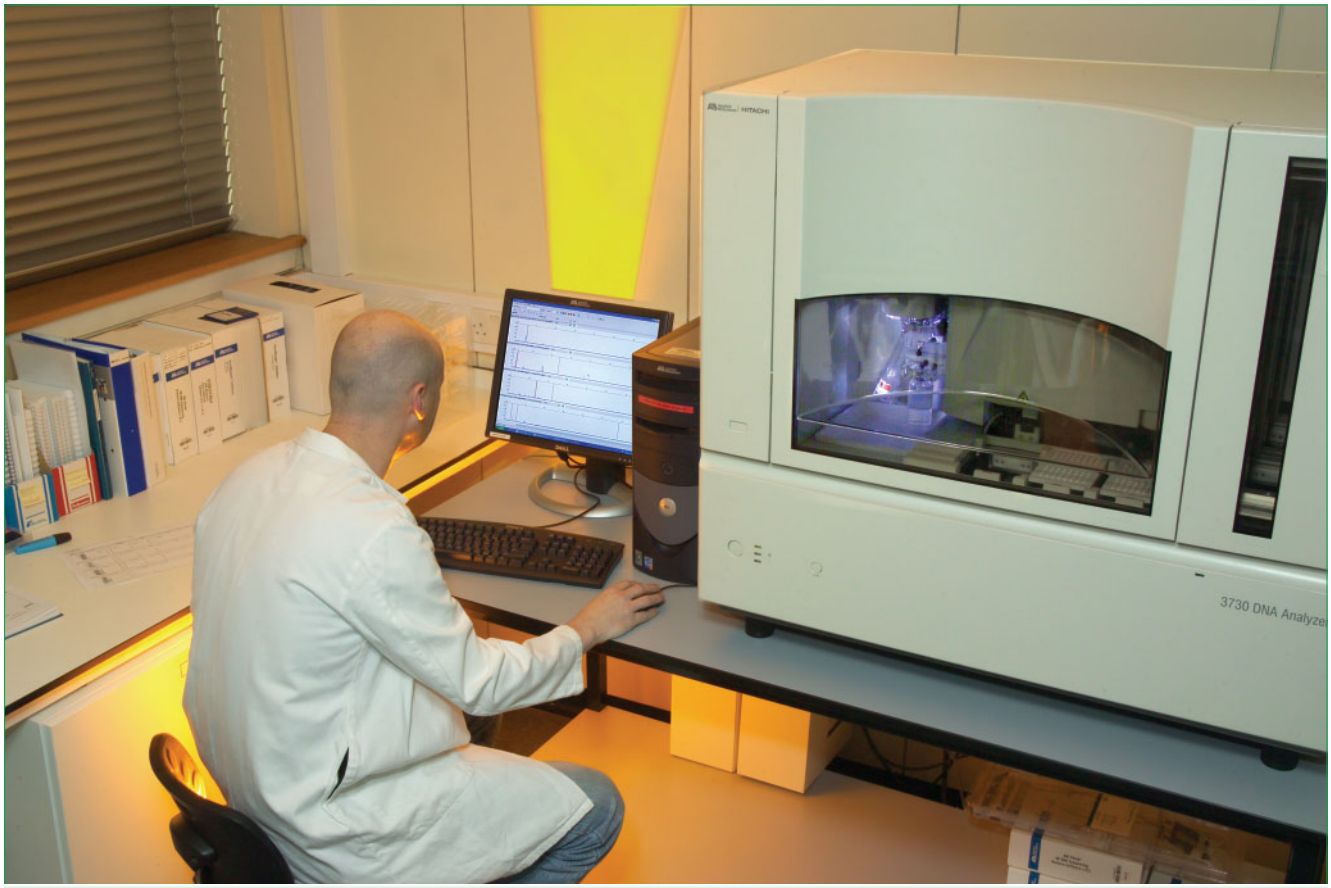


Figure 1 A) Genes are transcribed into precursor mRNAs which through AS give rise to different mRNAs and proteins with different functions. B) Developmental cues and external stimuli cause changes in transcription and alternative splicing resulting in altered splicing factor concentrations which further affect expression of downstream genes to alter the cell's growth or response properties.



regulatory factors which determine transcriptional and alternative splicing activity.

The challenges are to identify AS events, determine the functional consequences, and decipher the cellular code of regulatory proteins and signalling pathways responsible for altering AS patterns of target genes. As a first step, we have successfully developed a system to analyse AS in around 100 genes simultaneously. More than one third of the genes analysed show statistically significant changes in AS under different growth conditions and in different plant organs. Expansion of this system to cover a few hundred genes specifically involved in transcription, splicing and signalling in *Arabidopsis* and barley will allow us to investigate changes in AS in response to biotic and abiotic stresses, different growth conditions and developmental stages, and to link these changes with changes in transcription levels. This research is partially supported by a European Network of Excellence on AS and human disease (EURASNET) where 35 laboratories from 13 countries are analysing AS in yeast, plants, animals and humans to generate new knowledge of the mechanisms of AS regulation as a prerequisite to understanding a range of diseases and developing treatments.