Complex- Multilevel regulation of gene expression in plants: a general overview

Dr. Sritama Mukherjee *Assistant Professor, PG Department of Botany, Bethune College*
Dr. Sonali Sengupta *Research Associate, School of Plant, Environment and Soil Sciences, Louisiana State Univ. Agri. Cent., L.A.*
Prof. Arun Lahiri Majumder *Division of Plant Biology, Bose Institute*

Abstract
The process of gene expression in eukaryotes is complex and is regulated at multilevels. The pattern of expression may be tissue, developmental stage or environment specific. Environmental factors in turn may be biotic or abiotic. Stress response works in a highly coordinated manner where different signaling molecules and genes interplay with each other resulting in a signal transduction pathway. There are distinct levels of gene expression in eukaryotes viz. transcription, post transcription, translation and post translation where regulatory mechanism act. Recently unique regulatory mechanisms has been discovered which are involved in rerouting the faulty transcripts to avoid unnecessary translation. In this article, an attempt has been taken to review the different molecular mechanisms that regulate the expression of mostly 'regulatory' genes in higher plants at different levels of gene expression.

Key words: Gene expression, transcription, post-transcription, translation, post-translation

Introduction

Gene expression is the process by which information from a gene is utilized to synthesize a functional gene product i.e. mRNA, proteins or metabolites. Each eukaryotic cell expresses only a small percentage of the genes it contains at a given time point. Some genes are '**house-keeping**' that are constitutively expressed in all cell types and are involved in the basic metabolic processes.

Other genes, known as '**regulatory**' may express in selected cell types. In eukaryotes the gene expression patterns are complex, dynamic and differentially controlled, for instance, an expression profile may be: **Tissue specific, Developmental stage specific, External environment specific.**

Plant growth and development depends on the coordination of gene expression in a tissue-, temporal-, or signal-dependent manner. Often, the complex expression pattern observed for a given gene derives from regulation at the transcriptional and post-transcriptional levels. Such multi-level gene control is common in plants and needed to make the higher plants more adaptable and efficient living photosynthetic system.

Examples of tissue specific gene expression pattern in plants

Plant growth conditions have a significant effect on the differential gene expression patterns in cells or tissues. It is important to know when and where a gene is expressed in a plant. With the advent of '**reporter gene systems**' (GUS, GFP), '**tissue-specific promoters**' and '**plant transformation technology**' the cell-specific gene expression studies become easier in plants, and we can monitor the transcription of a gene under different conditions. For example, S-adenosylmethionine synthetase is involved in the biosynthetic pathway of polyamines and ethylene, catalyzing the biogenesis of S-adenosylmethionine. *Sam-1* is highly expressed in vascular tissues (xylem and phloem) which is confirmed by the GUS reporter gene assay in transgenic *Arabidopsis* plants (Peleman *et al.,* 1989). In maize, the endosperm-specific seed-storage proteins called zeins are encoded by a large multigene family. Histochemical analysis of transgenic tobacco is used to confirm the Zein promoter directed transient GUS expression in the endosperm (Scherntaner *et al.,* 1988).

Cell-specific gene expression studies have a large impact on the present agricultural scenario. Glutamine Synthetase (GS) functions as the major assimilatory enzyme for ammonia produced from nitrogen fixation, and nitrate or ammonia nutrition, also reassimilates ammonia released as a result of photorespiration and the breakdown of proteins and nitrogen transport compounds. GS isozymes are expressed in different cell types, tissues along with different subcellular localizations (chloroplast and cytoplasm) (Edwards *et al.,* 1989) the analysis of which largely helped in raising herbicide resistant crop plants (Oxtoby *et al.,* 1990).

Examples of developmental stage specific gene expression pattern in plants

Developmental stage specific gene expression includes both **temporal** and **spatial** expression patterns. The metabolic enzymes associated with C4 metabolism have been used previously as cell-specific markers to analyze developmental pattern in maize leaves (Mayfield and Taylor, 1984). The gene(s) for Rubisco (Ribulose-1,5-bisphosphate carboxylase) and NADP-ME (NADP-malic enzyme) are expressed specifically in bundle sheath cells in the presence of light whereas other C4 genes encoding PEPCase (phosphoenolpyruvate carboxylase) and NADP-MDH (NADP-malate dehydrogenase) expressed in mesophyll cells. *In situ* hybridization and cell separation experiments showed the cell-specific expression pattern of the above enzymes and demonstrated that these occur at an early stage of development. Rubisco LSU (large sub-unit) and SSU (small sub-unit) genes are found to be expressed in a ring surrounding the provascular cells prior to the differentiation of bundle sheath cells in developing maize leaves (Langdale *et al.,* 1988).

Crassulacean acid metabolism (CAM), a water-conserving photosynthetic pathway, involves a temporal separation of an *in situ* biochemical function as compared with the anatomical specialization and spatial separation of biochemical function in C_4 plants. Transcription abundance for the phosphoenolpyruvate carboxylase kinase (PPCK) which controls the phosphorylation state of PEPC is the only CAM-related enzyme for which a diurnal oscillation pattern was reported in constitutive CAM plants - *Kalanchoe fedtschenkoi* (Hartwell *et al.,*1999), *Opuntia ficus-indica* (Mallona *et al.,*2011) and in facultative CAM plant *Mesembryanthemum crystallinum* (Taybi *et al.,*2000) and is proposed to be the key clock system responsible for circadian regulation of CAM metabolism (Hartwell *et al.,*1999; Wyka *et al.,*2004).

Environment specific gene expression pattern in plants

As plants are bound to places, they have to be considerably more adaptable to stressful environments than animals. Environmental factors can be of abiotic and biotic nature.

Biotic environmental factors includes:

- infection from bacteria, fungi, viruses, nematodes or insects
- mechanical damage by herbivory
- effects of symbiosis

Abiotic environmental factors includes:

- temperature,
- humidity,
- light intensity,
- abundance and availability of water, minerals, and CO_2

Many other influences, which are only rarely beneficial to the plant includes wind (as distributor of pollen and seeds), or not beneficial or are even damaging (ionizing rays or pollutants), are also classified as abiotic factors.

Deviations from the physiological normal type are regarded as the conditions or situations for which we use the term **stress**. Hence the environmental factors deviating from the optimal intensity or quantity for the plant are called **stress factors**.

Biotic and Abiotic stress response in plants involves differential gene expression

Being sessile, plants have developed specific mechanisms that allow them to detect precise environmental changes and respond to complex stress conditions, minimizing damage while conserving valuable resources for growth and reproduction. The group of defense genes that are involved in different signaling cascades converge with a degree of overlap in the response programs for pathogen defense and abiotic stress protection. Emerging evidence revealed several molecules, including transcription factors and kinases, as promising candidates for common players that are involved in crosstalk between stress signaling pathways.

Stress response is a coordinated action of membrane, several signaling molecules and different genes that cross-talk with each other during the signal transduction process. Once triggered by an environmental challenge, plant cells

trigger a network of signaling events starting with stress perception at the membrane level, followed by signal transduction steps and ending with a cellular response **(Fig.1)**.

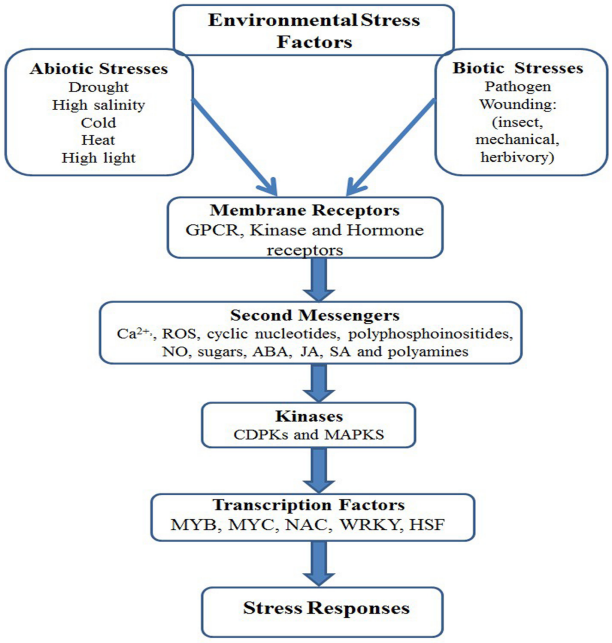

Fig.1. A schematic representation of different steps of a signal transduction pathway showing the cross-talk between abiotic and biotic stress responses (Adapted from Fujita *et al.,* 2006 and Atkinson and Urwin, 2012).

Multi-level gene regulation mechanisms in plants

In eukaryotes, gene expression is regulated at a number of distinct levels. There are regulatory systems for the control of transcription, precursor-RNA processing, transport of the mature RNA out of the nucleus, translation of the mRNAs, degradation of the mature RNAs and degradation of the protein products **(Fig.2).**

Levels of Regulatory mechanisms

1. Transcriptional control

Control of transcriptional initiation is the primary means used to regulate gene expression in eukaryotic organisms. Eukaryotic protein coding genes contain both **promoter** and **enhancer elements**. Promoter elements in the promoter proximal region, binds specific regulatory proteins that, in turn binds with enhancer elements and activate transcription. **Remodelling of chromatin** plays an important role to activate transcription which is brought about by the binding of activators to enhancers. The activators recruit chromatin remodelling complexes, either a type that acetylates nucleosomes (Histone acetyl transferases, HATs) or another that moves or restructures nucleosomes allowing the transcription machinery to access the promoter.

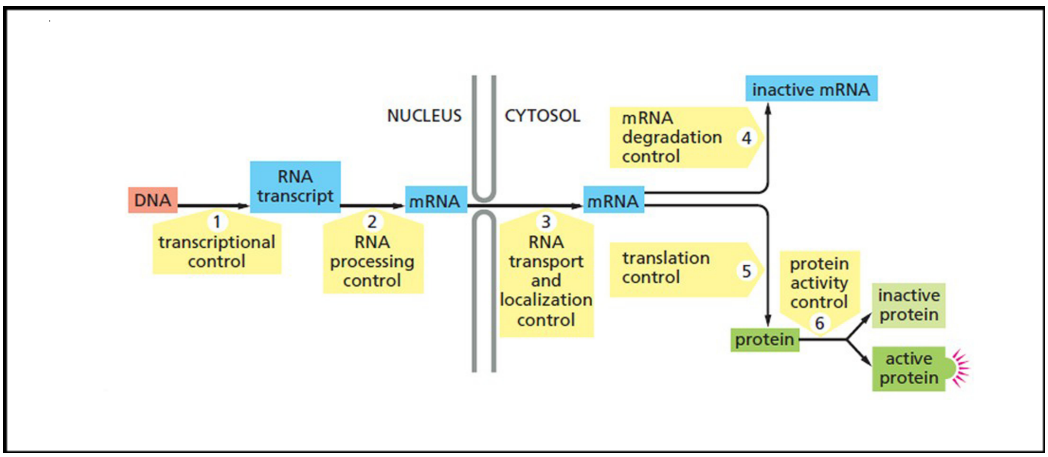

Fig.2. Different levels of control of gene expression in eukaryotes.

2. Post-transcriptional control

Many examples exist where changes in the rate of synthesis of a particular protein occur without a corresponding change in the transcription rate of the corresponding gene.

(a) RNA processing control

It regulates the production of the mature RNA molecules from precursor-RNA molecules.

Alternative splicing

Alternative splicing (AS) creates multiple mRNA transcripts, or isoforms, from a single gene. Four main types of alternative splicing are known: exon skipping, alternative 5' and 3' splice sites and intron retention **(Fig.3)**. Exon skipping is the most frequent and intron retention the rarest alternative splicing form in animals (Kim *et al.,* 2007), while intron retention is the most common alternative splicing in *Arabidopsis* and rice (>50%; Kim *et al.,* 2007, Ner-Gaon *et al.,* 2004). Use of alternative splice-sites in the same pre-mRNA (selective utilization of multiple splice sites) allows the synthesis of different proteins from the same gene or can function as an "on-off switch" to make or not make a functional protein. Alternative splicing is known to occur in a tissue-specific or developmental fashion. The characterization of spinach and *Arabidopsis* ribulose bisphosphate carboxylase/oxygenase (Rubisco) activase is the first demonstrations of AS in plants (Werneke *et al.,*1989). Rice orthologs of 40% of the *Arabidopsis* genes were demonstrated to alternatively spliced isoforms (Wang and Brendel, 2006).

Alternative polyadenylation

Alternative polyadenylation generates different transcripts with altered coding capacity for proteins and/or RNA. The exact molecular mechanism is still unknown. Over 50% of plant genes studied possess multiple APA sites in their transcripts which are used extensively to generate diversity in their transcriptomes, the best-known ones are related to flowering time control pathways and stress responses (Xing and Li, 2010).

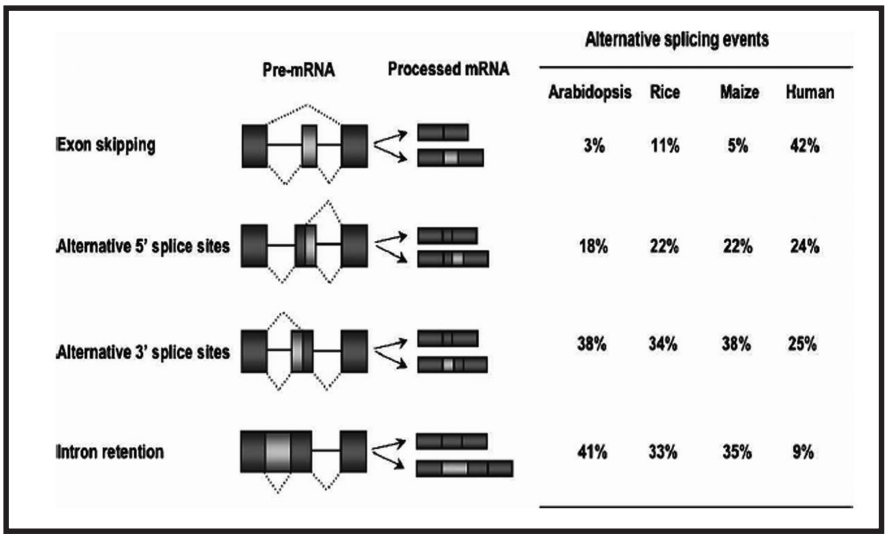

Fig.3. Common types of Alternative splicing and their occurrence in different organisms (Adapted from Barbazuk *et al.,* 2008).

(b) mRNA transport

The transport of mRNAs from the nucleus to cytoplasm is another check point and proteins are needed here to facilitate the exit of mature mRNAs. Spliceosome signals retain the premature RNA in the nucleus until intron removal occurs. Thereafter the free, mature, capped mRNA can interact with nuclear pore complex and exit.

(c) mRNA stability

Once in the cytoplasm, an approach used by the cell to quantitatively regulate output of a gene is to modulate the

stability of an mRNA which depends on its rate of transcription and turn over. mRNAs vary greatly in their stability, with half-lives ranging from a few minutes to more than a day, in response to developmental and environmental stimuli such as nutrient levels, hormones, viruses, and temperature shifts. An example is the pea (*Pisum sativum*) ferrodoxin (*Fed-1*) mRNA, whose stability is regulated by light.

Several pathways have been identified in yeast, the most important of which is the **Deadenylation-Dependent Decapping Decay pathway**. Components of this pathway have been identified in plants but the mechanism is not demonstrated well. A **Downstream element** (DSE), was identified in the small auxin-up RNA transcript (SAUR) in Arabidopsis, found to confer instability on a number of plant genes. **AU-rich elements** (AREs) are present in the 3' UTR of many mRNAs and are potent sequence elements for post-transcriptional regulation of gene expression. AREs influence the stability or translation of a given mRNA usually through binding of ARE-specific RNA-binding proteins (Barreau *et al.,* 2005). **MicroRNAs** (miRNAs) and **small interferring RNAs** (siRNAs) are riboregulators that causes post-transcriptional gene silencing (PTGS) in plants through **RNA interference** (RNAi) mechanism. There are 15 distinct miRNA families in *Arabidopsis* which are also conserved in rice (Jones-Rhoades and Bartel, 2004).

3. Translational and Post translational control

Global translation regulation in eukaryotes is generally achieved by the modification of **eukaryotic initiation factors** (eIFs), several of which (e.g. eIF4E and eIF2) are phosphoproteins (Day and Tuite, 1998). For example the altered translation rates in potato tubers during both wounding and hypoxia appear to be regulated by elongation factor 1 subunit á (EF-lá) (Morelli *et al.,* 2004). Translational control of individual mRNAs often depends upon the structural features of the transcript itself, and may include structures in the 5' UTR that inhibit initiation directly, for example by impeding 40S subunit binding or scanning, or indirectly, by acting as receptors for a regulatory RNA binding protein. The ribosome itself can also be targeted to exert translational regulation and several of its protein constituents can undergo post-translational modifications (Day and Tuite, 1998).

Post translational modifications (PTMs) are covalent processes that greatly increase protein complexity and dynamics, co-ordinating the intricate regulation of biological events (Kwon *et al.,* 2006). They modify the primary structure of proteins in a sequence-specific way that includes the reversible addition and removal of functional groups by phosphorylation, acylation, glycosylation, nitration, and ubiquitination (Mann and Jensen, 2003; Seo and Lee, 2004). These modifications induce structural changes in proteins and modulate their activities like subcellular localization, stability, and interactions with proteins and other molecules (Kwon *et al.,*2006). In addition to ubiquitin several other polypeptide tags have emerged in plants that act as chemical modifiers for the posttranslational control of proteins that affect growth, development and homoeostasis. The list includes RUB-1 (related to Ub-1; also known as NEDD8), SUMO (small Ub-like modifier), ATG-8 (autophagy-8) and ATG-12, UFM-1 (Ub-fold modifier-1) and HUB-1 (homology to Ub-1) (Downes and Vierstra, 2005).

Unique Regulatory mechanisms

There are translation dependent mechanisms in the cell to detect and subsequently degrade aberrant mRNA transcripts thereby protecting the cell from potentially toxic protein products and preserving translation fidelity.

(a) Non-stop and No-go decays

Non-stop decay (NSD) targets mRNAs that lack a stop codon that can be generated by breakage, or by the absence of an in-frame stop codon, causing translation to proceed along the poly(A) tail. Premature polyadenylation might be an important contributor to the production of substrates for this decay pathway and is conserved in mammals and yeast (Garneau *et al.,* 2007).

No-go decay (NGD) is recently discovered in yeast which prevents the sequestration of translation factors to faulty transcripts by detecting stalled ribosomes on an mRNA and endonucleolytically cleaving the mRNA near the stalled site (Garneau *et al.,*2007).

(b) Non-sense mediated decay (NMD)

NMD detects and degrades transcripts containing premature termination codons (PTCs) during the pioneer round of translation that may arise from mutations, frame-shifts, intron retention, inefficient processing, leaky translation initiation and extended 32 UTRs **(Fig.1.5)**. It is proposed that NMD may have a proofreading role in gene expression, eliminating transcripts that have not been spliced owing to their suboptimal splice signals (Sayani *et al.,* 2008; Jaillon *et al.,* 2008) and also regulates alternative splicing by eliminating unproductive splice variants that contain PTCs (McGlincy and Smith, 2008).

The detection and degradation of prematurely terminating transcripts coupled to repressed splicing can be described as a form of regulated unproductive splicing and translation (RUST) achieved by means of NMD (Green *et al*., 2003). Three well-investigated *trans*-acting factors in NMD are the proteins encoded by the *UPF1*, *UPF2* and *UPF3* genes, discovered in *Saccharomyces cerevisiae* (Culbertson *et al.,*1980) and a plethora of additional factors (SMG1, SMG5, SMG6 and SMG7) were also characterized (Conti and Izaurralde, 2005). NMD in mammals is characterized by an **'Exon Junction Complex'** (EJC) which is deposited 20–24 nucleotides upstream of every exon junction, usually displaced by translating ribosomes.

In mRNAs containing PTC, EJC remains inappropriately associated the mRNA downstream of the PTC, where it is detected by the surveillance machinery (Brogna and Wen, 2009; Garneau *et al.,* 2007). In yeast NMD is described by a **'Faux 3'UTR'** model which says that premature translation termination is intrinsically abnormal because it takes place a long distance from the 32 end, and that this prevents the normal interaction between the terminating ribosome and poly(A) binding protein (PABP) (Brogna and Wen, 2009).

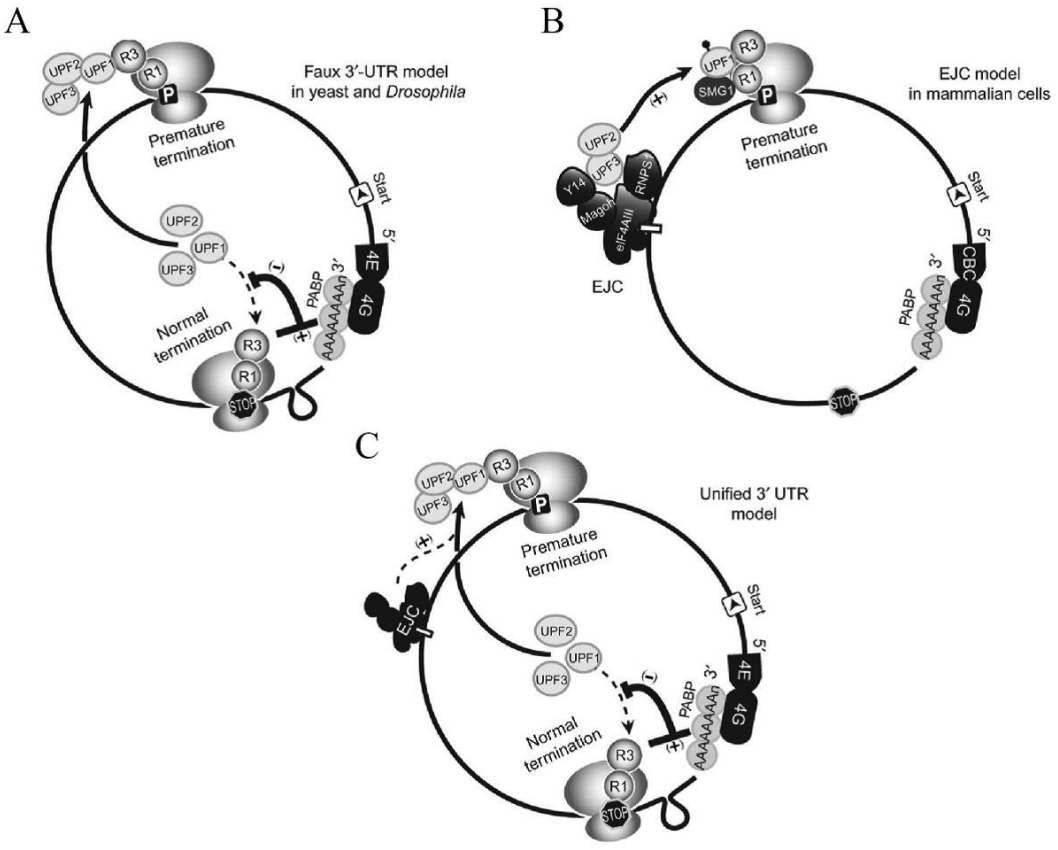

Fig.4. Current NMD models. (A) The faux or false 32 UTR model: R1 and R3 represent peptide-release factors eRF1 and eRF3; 4E and 4G represent translation-initiation factors eIF4E and eIF4G. (B) The EJC model: The line with a black dot denotes phosphorylation of UPF1. CBC indicates the cap binding complex. (C) The unified faux 32 UTR model (Adapted from Brogna and Wen, 2008).

A new NMD model has been proposed by Brogna and Wen, 2009 termed as **'Unified Faux 32 UTR'** model which combines the above two models depicting the role of both EJC and PABP in recognition of PTC and thereby degradation of mRNA **(Fig.4)**. NMD is least characterized mRNA degradation process in plants **(Table.1)**.

Table.1. NMD in Plants.

Gene	Plant	References
Kunitz trypsin inhibitor (*Kti3*)	*Glycine max*	Jofuku *et al.,* 1989
Phytohemagglutinin (*PHA*)	*Phaseolus vulgaris*	Voelker *et al.,* 1990
Small-auxin-up-RNA(*SAUR-AC1*), Auxin resistant (*AUX1*)	Arabidopsis	Gil and Green, 1996; Marchant and Bennett, 1998
Chalcone synthase (*Chs*)	Petunia	Que *et al.,*1997
Magnesium chelatase (*Xantha-f*)	*Hordeum vulgare*	Gadjieva *et al.,* 2004
Ferredoxin1(*Fed1*)	*Pisum sativum*	Dickey *et al.,* 1994; Petracek *et al.,* 2000
Starch synthase mutant(*Wx-A1*)	Wheat	Saito and Nakamura, 2005
Starch synthase(*Waxy*)	Rice	Isshiki *et al.,* 2001

Conclusion

One of the major challenges faced by experimental biologists today is to understand the genetic and environmental factors regulating differential gene expression in plants. Over the last fast years there has been a significant progress in this area of research may. In the present paper an initiative has been taken to review the regulatory mechanisms that control gene expression at transcriptional, post-transcriptional, translational and post-translational levels. There are recently discovered processes like non-stop, no go and nonsense mediated decays that are prominent in yeasts and reports are few in case of higher plants. This article may provide an insight into the details of this mechanisms which are also helpful to understand how different biotic and abiotic stresses control gene/protein expression and their molecular details.

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