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Session 2
RNA turnover and surveillance

Lecture 2.1

Linking mRNA methylation and 3′ end processing to development

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Genetic screens for mutations affecting *Arabidopsis thaliana* flowering time have recurrently identified proteins that affect mRNA 3′ end formation. For example, my lab revealed that the RNA binding protein encoded by *FPA* (one of the original late flowering mutants identified by Maarten Koornneef) controls poly(A) site choice and transcription termination. We have recently used transcriptome-wide single molecule RNA sequencing to assess how widespread the impact of FPA on 3′ end formation is and in doing so, linked FPA to specific sub-sets of gene targets and to processes other than flowering time control. In order to understand the mechanism by which FPA controls RNA 3′ end formation we have now developed an *in vivo* interaction proteomics procedure, which identifies closely associated proteins in living plant cells. Interestingly, this proteomics approach led us to study mRNA methylation. I will present our latest findings that link mRNA methylation and 3′ end formation to development.

Lecture 2.2

Unique features of the plant RNA exosome

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RNA exosomes are present in both nuclear and cytoplasmic compartments of all eukaryotic cells and participate in 3′-5′ processing, surveillance and turnover of both coding and non-coding RNAs. Exosomes are multimeric complexes composed of a nine-subunit core complex (Exo9) which interact with RNases and activators/adapters such as RNA helicases, RNA binding proteins and non-canonical poly(A) polymerases. The structural organization of the core exosome (Exo9) is conserved across eucaryotes: six subunits related to bacterial RNase PH form a ring-like structure, that is capped by three subunits with RNA binding domains. Despite this common organization, Exo9 from plants differs remarkably from other eukaryotes. In both yeast and humans, Exo9 has lost its catalytic activity and both endo- and exoribonuclease activities are conferred by associated RNases. By contrast, we show that Exo9 purified from *Arabidopsis thaliana* has a phosphorolytic activity. AtExo9 has also unique catalytic features as compared to other phosphorolytic enzymes such as the bacterial RNase PH, PNPases or the archaeal exosomes. Interestingly, all residues critical for the phosphorolytic activity of AtExo9 are conserved in the green lineage, suggesting an important function in plants. Another interesting feature of plant RNA exosomes is the complexity of associated co-factors. In both yeast and human, all nuclear functions of the exosome require the RNA helicase MTR4. Our data indicate that the Arabidopsis core exosome can associate with two related RNA helicases, AtMTR4 and HEN2. Reciprocal co-immunoprecipitation shows that each of the RNA helicases co-purifies with the exosome core complex and with distinct sets of specific proteins. While AtMTR4 is a predominantly nucleolar protein, HEN2 is located in the nucleoplasm. The major role of AtMTR4 is the degradation of rRNA precursors and rRNA maturation by-products whereas HEN2 is involved in the degradation of a large number of polyadenylated nuclear exosome substrates. These substrates include snoRNA and miRNA precursors, incompletely spliced mRNAs, and spurious transcripts produced from pseudogenes and intergenic regions. Interestingly, HEN2 is conserved throughout green algae, mosses and land plants but absent from metazoans and other eukaryotic lineages. Our data indicate that, in contrast to human and yeast, plants have two functionally specialized RNA helicases that assist the exosome in the degradation of specific nucleolar and nucleoplasmic RNA populations, respectively.

Short talk 2.1

*N*6 adenosine methylation of mRNA – an expanding role

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*N*6-methyladenosine (m^6A) is a ubiquitous base modification found internally in the mRNA of most Eukaryotes. Levels of methylation equivalent to at least 50% of transcripts carrying m^6A are common, and most of this methylation appears to be associated with the 3′ ends of transcripts. In *Arabidopsis thaliana*, all tissues contain m^6A but levels vary between tissues and developmental stages. The methylase itself (MTA) as well as other components of the methylase complex responsible for m^6A formation are essential during embryonic development, and a reduction in m^6A levels during later growth stages gives rise to plants with altered growth patterns, changed cell identities and reduced apical dominance. A related methylase (MTB) may also be part of this complex but may have a dispensible function in certain vegetative tissues. The global analysis of gene expression from reduced m^6A plants show a dramatic increase in transcript abundance for messages indicative of various stress responses, and there is a preferential distribution of m^6A with polysome-associated transcripts under certain stress conditions.

Short talk 2.2

Functional specialization of *Arabidopsis* poly(A) polymerases in relation to flowering time and to abiotic stress

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Polyadenylation of pre-mRNAs is a critical process in eukaryotic gene expression. The *Arabidopsis* genome encodes three nuclear poly(A) polymerases. Despite their ubiquitous expression pattern at all developmental stages and their general enzymatic function of polyadenylating mRNAs, the three poly(A) polymerases show differentiation of function, as assessed by their mutant phenotypes (Vi et al., 2013). One knockdown leads to an early flowering phenotype and specific growth defects in the plant, while knocking out the other two poly(A) polymerases causes a delay in flowering time. To unravel the function of poly(A) polymerases in the regulatory network controlling the onset of flowering, I am using several approaches. An analysis of the transcriptome of mutant seedlings and flowers by RNAseq revealed profound changes at the transcriptional level in *poly(A) polymerase* (*paps*) mutants. Crosses between *paps* mutants and flowering time mutants are being carried out. To reveal the molecular mechanism underlying the *paps* mutant flowering time phenotype, the poly(A) tail lengths and expression levels of RNAs involved in flowering time control are being examined under different growth conditions. Recently, the *paps* mutants have been revealed to exhibit opposite responses to a set of diverse stress treatments. To broaden our understanding of the importance of polyadenylation in the plant reaction to stress, I am particularly analysing the *paps* mutant response to oxidative stress. Interestingly, a different polyadenylation factor mutant has been shown to be resistant to oxidative stress before (Zhang et al., 2008). Stress-related genes are being examined for changes in poly(A) tail lengths in the *paps* mutants. The detailed analysis of the differential *paps* mutant phenotypes adds important evidence to the novel concept of gene expression regulation based on poly(A) polymerase-specific mRNA polyadenylation control.

Lecture 2.3

Mechanism and evolution of the autoregulatory circuit that controls the expression of eukaryotic release factor 1 (eRF1) in plants

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When a translating ribosome reaches a stop codon three things, normal translation termination, translational readthrough (RT) or Nonsense-mediated mRNA decay (NMD) can happen. The outcome depends on the stop context and the level of the eRF1 and eRF3 termination factors. At normal stop context, the eRF1 binds to the stop codon and terminates translation. RT occurs when the stop context does not favor the binding of eRF1 to the stop. Instead the stop codon is bound by a near cognate tRNA and elongation is continued till the next in-frame stop codon. If the 3′UTR contains NMD cis elements (unusually long 3′UTR or an intron in the 3′UTR), the translation will be slow and NMD is induced. NMD does not interfere with the peptide release but trigger the rapid decay of the target mRNA. In bacteria, the expression of the RF2 termination factor, which functions similarly to eRF1, is strictly controlled by an autoregulatory circuit. High RF2 level leads to early termination of RF2 translation and to the accumulation of an inactive, truncated protein, while low RF2 level stimulates the expression of the active termination factor. Although several lines of evidence support that eRF1 expression is tightly regulated in eukaryotes, the mechanism of eRF1 control is not known. We found that in plants, eRF1 level plays a critical role in all three termination codon related events, it intensifies normal termination, reduces the frequency of RT and stimulates NMD. We show that in plants an elegant regulatory system has evolved that controls the eRF1 level via RT and NMD, thereby ensuring the balance among normal termination, RT and NMD.

Short talk 2.3

Alternative splicing and nonsense-mediated decay in a null mutant of *UPF1* in Arabidopsis

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We previously showed that around 18% of genes in Arabidopsis potentially undergo AS/NMD using a high resolution RT-PCR platform and mutants in the NMD protein genes, *UPF1* and *UPF3* (Kalyna et al., 2012). The *upf1-5* and *upf3-1* mutants are viable but the *upf1-3* mutant is seedling lethal. Disruption of the UPF1 gene in *upf1-3* causes changes in gene expression and AS profiles, increased salicylic acid levels and expression of PR genes giving a hypersensitive defence response (HR) (Riehs-Kearnan et al., 2012) suggesting that one function of NMD is regulation of plant defence responses. By combining a mutation in the *PAD4* gene involved in the salicylic acid pathogen signalling pathway, the lethality of *upf1-3* has been overcome (Riehs-Kearnan et al., 2012). The *upf1-3 pad4* double mutant now allows the real extent of NMD on transcripts to be determined in a null background. We have analysed alternative splicing/NMD in the *upf1-3 pad4* mutant and a related double mutant, *smg7-1 pad4,* by high resolution RT-PCR using primers targeted to known NMD-sensitive transcripts to investigate the effect of the mutants on NMD. We found large significant changes in the level of NMD-sensitive alternatively spliced products in the mutants and particularly in the *upf1-3 pad4* mutant background. The increases were often far greater than observed in the weaker *upf1-5* and *upf3-1* mutant alleles tested previously suggesting that substantial proportions of transcripts from some genes are degraded and effectively turned over by NMD. We also identified novel NMD-sensitive transcripts and further demonstrated that many transcripts with unspliced introns are not turned over by NMD. We are currently examining whether stabilised NMD-sensitive transcripts in the mutants are translated using a novel plant SILAC method (Lewandowska et al., 2013).

Short talk 2.4

Analysis of the stress triggered mRNA decay process in *Arabidopsis thaliana*

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To survive adverse and ever changing environmental conditions, an organism must be able to adapt. It has long been established that the cellular reaction to stress includes the upregulation of genes coding for specific stress-responsive factors. We demonstrate in the present study that during the early steps of the heat stress response, 25% of *Arabidopsis* seedlings transcriptome is targeted for rapid degradation. Our findings demonstrate that this process is catalysed from 5′ to 3′ by the cytoplasmic exoribonuclease XRN4 which function is seemingly reprogrammed by the heat-sensing pathway. The bulk of mRNAs subject to heat-dependent degradation is likely to include both the ribosome-released and polysome associated polyadenylated pools. The co-translational decay process is facilitated at least in part by LARP1, a heat specific cofactor of XRN4 required for its targeting to polysomes. Commensurate with their respective involvement at the molecular level, LARP1 and XRN4 are necessary for the thermotolerance of plants to long exposure to moderately high temperature with *xrn4* null mutants being almost unable to survive. These findings provide for the first time mechanistic insights regarding a massive stress-induced post-transcriptional downregulation and outline a potentially crucial pathway for plant survival and acclimation to heat stress.

Short talk 2.5

Insights into translational regulation of thermal stress response of *Arabidopsis thaliana* by ribosomal profiling

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Translation provides an additional level to regulate expression of the protein coding genes. It allows for a higher plasticity of plant organisms to response to environmental conditions or internal development signals. Ribosome profiling coupled with RNA sequencing enables assessing in depth the regulation at the post transcriptional and translational level. It combines the power of global measurements of steady state levels of transcripts with the sequencing of the ribosome-protected fragments of mRNA, which in turn is informative on which mRNAs are being actively translated and on the dynamics of this process. Here we apply ribosome profiling in order to assess transcriptional and translational response of plants growing in the sub-lethal heat stress, compared to optimal growth conditions. The interest on studies on the influence of increased temperature effect on plants is rising because of the global warming effect: the vegetation zones are shifting pole wards and more and more areas are already experiencing temperatures which are suboptimal or even detrimental to the plant growth and development. Our data indicates that heat stress in plants leads to global downregulation of the translation processes, however not all of the genes seem to be affected. We identified groups of genes whose biosynthesis rates are not affected by stress-regulated downregulation of translation; they show even higher synthesis rate compared to optimal growth conditions. Most of these proteins are known to be involved in the stress response, however we identified several previously uncharacterized protein which can potentially participate in these processes. Our data add another twist to the discussion whether non-coding RNAs are translated or not. Upon heat stress, some non-coding RNAs are upregulated on transcriptional level, however most of them, with few exceptions, were not detected in the ribosome protected fragments datasets. In summary, our data shows a detailed view on the heat stress response in plants, both on transcriptional and translational levels and provide numerous interesting points, which will be followed in our ongoing research.