Progress Article

Protein Turnover in Plant Biology

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The protein content of plant cells is constantly being updated. This process is driven by the opposing actions of protein degradation, which defines each polypeptide's half-life, and protein synthesis. Our understanding of the processes that regulate protein synthesis and degradation in plants has advanced significantly over the last decade. Post-transcriptional modifications that influence features of the mRNA populations, such as poly-A tail length and secondary structure, contribute to the regulation of protein synthesis. Post-translational modifications such as phosphorylation, ubiquitination and non-enzymatic processes such as nitrosylation and carbonylation, govern the rate of degradation. Regulators such as the plant TOR kinase, and effectors such as the E3 ligases, allow plants to balance protein synthesis and degradation under developmental and environmental change. Establishing an integrated understanding of the processes that underpin changes in protein abundance under various physiological and developmental scenarios will accelerate our ability to model and rationally engineer plants.

Thousands of different proteins make up the machinery of plant cells. Many biological phenomena influence the rate at which these proteins are synthesized (k_s) and degraded (k_d). Today, large volumes of data are collected that allow the measurement of transcriptional^{8,10}, post-transcriptional^{1,4}, and translational events^{2,3}, all of which contribute to k_s . The processes that contribute to k_d - a protein's location, compartment specific proteases/peptidases, its function, and protein interactors^{6,7,11,12} - are also amenable to large-scale experimental verification. Furthermore, the proteomic complement of a tissue, and how this changes over time, can now be measured quite easily; proteomics routinely provides information on the abundance of thousands of proteins in a single experiment¹³⁻¹⁵. When abundance data is combined with information on k_s and k_d , it is possible to deduce whether changes in abundance result from one or both of these processes. By combining these datasets and approaches we can develop a better understanding of the factors that contribute to each protein's abundance. Here, we review the cellular processes that contribute to protein synthesis and degradation, and their sensitivity to developmental and environmental change.

Protein abundance: multiple mechanisms of regulation

Proteins are synthesized by the action of ribosomes in the cytosol, plastid, and mitochondrion (Figure 1). Although most of the translation occurs in the cytosol, a large fraction also occurs in the plastid of photosynthesizing tissues³. Translation initiation in the chloroplast is regulated by light and redox chemistry and is quickly inhibited upon the initiation of darkness. In contrast, translation in the cytosol seems to be limited by carbon reserves. And mitochondrial translation appears to be relatively continuous³. These broad trends likely reflect the cyclic availability of ATP in the different compartments. Protein synthesis is the single most costly process in the cell and even in extreme environmental conditions its regulation is strictly maintained⁹. Under some circumstances, regulation is transcriptional in nature, and determined by signaling cascades¹⁰ or even through changes to the translational machinery itself^{10,16}. In many cases, features of the mRNA populations, such as secondary structure and poly-A tail length, can influence synthesis rates^{1,2,4,17}, providing clear evidence of post-transcriptional modulation of this process.

Outside of the organelles, protein degradation occurs largely through the ubiquitin proteasome system (UPS). Located in the cytosol and the nucleus, the activity of this system is linked to hormonal signaling and life stage transitions^{18,19}. The UPS consists of a series of enzymes (E1, E2 and E3 ligases) that tag proteins destined for degradation with ubiquitin. Multiple cycles of ubiquitination lead to the formation of a poly-ubiquitin chain, typically the signal for degradation via the proteasome. The ubiquitin-

activating E1 enzyme charges the ubiquitin molecule and transfers the activated form to the ubiquitin-conjugating E2 enzyme. The E2 enzyme attaches the activated ubiquitin to the target protein, with target recognition provided by the ubiquitin-ligating E3 enzyme. In some cases conjugation is conducted by the E3 enzyme. Proteins tagged with poly-ubiquitin tails are degraded in the proteasome, a large protein complex composed of a 20S core and two regulatory 19S lids (Figure 1). The composition of this complex can change under biotic stress, providing plants with some degree of flexibility in the rate and extent of protein degradation²⁰. In *Arabidopsis*, there are 2 E1 loci, approximately 40 E2 genes, and more than 1400 predicted E3 ubiquitin ligase genes²¹. Given their number, it is believed that E3 ligases - each supposedly providing a different collection of targets for degradation - provide much of the specificity needed to adapt to a myriad of abiotic stresses as well as developmental transitions¹⁹. The UPS also plays an important role in defending plants against biotic stresses, such as the degradation of virus movement proteins²¹.

The UPS does not just act on cytosolic proteins but also influences organelles in the cell. In *Arabidopsis*, the SP1 protein, a RING-type E3 ligase localized to the outer plastid envelope, facilitates de-etiolation. The ligase faces the cytosol and allows the plastid to interact with the UPS, promoting degradation of certain components of the plastid important machinery, and thereby chloroplast biogenesis¹⁸. Similarly, the ubiquitin protease UBP27, recently identified as an outer mitochondrial membrane protein in *Arabidopsis*, also faces the cytosol, allowing interaction with the ubiquitination system. Overexpression of this protease alters mitochondrial morphogenesis²², implying that ubiquitination at the mitochondrial surface is important for organelle function. Organellar proteins are also broken down by autophagy, a coordinated cellular process used to recycle various subcellular components and independent of the UPS. Final degradation occurs in the vacuole. This process is far more dynamic and adaptive than thought previously, and provides metabolic building blocks under various stress situations²³. Finally, organelle-specific proteases allow a finer control of specific processes in organelles, independent of the UPS^{7,24,25}.

A combination of transcriptional, post-transcriptional, translational, and post-translational mechanisms allows plants to balance and fine tune protein synthesis and degradation on a regular basis, in order to meet environmental⁹, developmental^{18,26} and energetic needs⁹. Although plant biologists have understood and appreciated this symmetry for quite some time, 'omics in combination with more targeted studies are beginning to reveal the details of these regulatory mechanisms on a scale not previously seen. A key advance in this respect has been establishing the role of regulators of protein synthesis and degradation, with an example being the plant homolog of the mammalian regulator mTOR (Figure 1). Although well known in non-plant systems for stimulating protein synthesis via phosphorylation of the translational activator S6 kinase, the transcription factor E2Fa, and regulation of ribosome abundance, the protein kinase mTOR was generally believed to repress protein degradation by phosphorylation of the autophagy related 1 protein (Atg13), which inhibits interaction of the ATG1/ATG13/ATG17 kinase complex components in yeast and ATG1 and ATG13 orthologs in mammals, and thereby autophagy^{27,28}. However, modification of mTOR activation in mammals suggests that mTOR upregulates proteasomal subunits and protein turnover, and therefore enhances both protein synthesis and degradation²⁹. Studies to date suggest that the plant TOR kinase stimulates protein synthesis through its impact on ribosome number and phosphorylation of S6 kinase, but there is a general reduction in the abundance of transcripts associated with protein degradation^{10,28}.

Plants have evolved to regulate protein synthesis and degradation during, and under optimal as well as extreme environments, emphasizing the importance and cost of these two processes and the need

for their coordination. For instance, the application of ^{35}S and 2DE at successive time intervals during seed germination in *Arabidopsis* has revealed that translation and degradation change in a coordinated fashion during germination²⁶. Environment-driven changes in protein synthesis can be seen in the response of anoxia and hypoxia tolerant rice varieties to oxygen deficits. In these plants, the fraction of energy allocated to protein synthesis was found to rise from 19 to 52% in the coleoptiles of flood-tolerant plants under low oxygen conditions, despite an overall reduction in ATP production and protein synthesis⁹. The up-regulation in the relative amount of energy allocated to protein synthesis presumably facilitates the rapid elongation of the coleoptile (i.e. the snorkel response) that allows these plants to cope with flooding⁹.

Key determinants of protein stability

The N-end rule, where the N-terminal amino acid determines a protein's stability, was once thought to explain a large proportion of the variance in the stability of eukaryotic proteomes³⁰. However, studies of protein turnover in plant and non-plant systems - which report measurements of k_d for hundreds to thousands of proteins^{25,29-32} - suggest that the turnover rates of many protein types cannot be explained by this simple rule. It is clear from these studies of protein turnover that the physical location of the protein, as well as protein-protein interactions, impact protein stability. Additionally, cofactor binding, and its catalytic consequences, can also influence the process^{7,11}. However, studies that average different cells do not capture the full complexity of the issue, as protein expression levels are known to be affected by tissue¹³ and cell type^{15,37-45}, and therefore whole-organ averages likely underestimate variations in protein stability for many proteins. Significant differences have also been observed at the subcellular and protein complex level.

In the only large scale assessment of k_s and k_d , the turnover characteristics of over 8,000 mammalian proteins were determined on a subcellular basis. Some of the proteins found in more than one compartment were found to exhibit different turnover rates. For instance, ribosomal proteins in the nucleolus possessed a shorter half-life than those in the cytoplasm¹². In plants, detailed analyses of mitochondrial complexes I and V have revealed significant differences in the stability of specific protein subunits when embedded in holocomplexes as opposed to complex assembly intermediates. The findings suggest that plant electron transport chain complexes have evolved a different order of protein assembly, that involves protein subunits not found in the mammalian electron transport chain^{5,46}. Similarly, the k_d of glycolytic super-complexes associated with the outer mitochondrial membrane in plants is slower than the whole-tissue average for the same enzymes³⁶, the stability of glutamate dehydrogenase varies with isoform⁴⁷, and protein-protein interactions in cryptochrome and phytochrome signalling pathways dictate the stability of photochrome interacting factors⁴⁸. The presence of cofactors can also alter the stability of proteins. For example, chlorophyll, present in light-grown tissues, stabilizes components of photosystem II⁴⁹, and proper insertion of FAD and Cu^{2+} increases the stability of ferreredoxin-NADP+-oxidoreductase⁵⁰ and plastocyanins⁵¹ respectively. Together these studies point towards the tremendous complexity of plant proteome turnover characteristics. To further our understanding of this complexity will require a combination of centrifugation, electrophoretic and chromatographic strategies, which will allow the adequate separation of the sub-populations of the thousands of protein isoforms and proteoforms, the different protein products of a single gene, in plant tissue¹².

The post-translational modification of a protein not only affects its location and protein interactors, but also impacts its stability (Figure 1). Like in many other areas of biology, research into the role of post-translational modifications on protein stability in plants has lagged relative to that in

mammalian and bacterial systems. Phosphorylation is an oft studied form of post-translational modification, and in plants is known to affect the stability of proteins such as the D1 and D2 proteins of photosystem II⁵². Phosphorylation of PSII core proteins also facilitates efficient transfer of damaged photosystem II monomers to stroma-exposed portions of the thylakoid membrane, where damaged D1 can be replaced⁵³. The importance of this modification for plant signalling, along with protein stability and synthesis, has been demonstrated in phosphoproteomic analyses in plants¹⁴. Large-scale analyses of the role of phosphorylation in protein stability in plants are yet to take place. However, the identification of a phosphatase, TOPP4, that dephosphorylates DELLA protein rendering it more susceptible to degradation, highlights the importance of the phosphorylation status of the DELLA proteins - which suppress gibberellin-dependent growth - for plant growth and development⁶.

Several reports have also begun to characterize the plant ubiquitylome and establish the role of ubiquitin based post-translational modifications for protein stability in plants, as well as other regulatory mechanisms. One proteomic investigation identified nearly 950 ubiquitinated proteins, involved in a wide range of biological activities, in *Arabidopsis* seedlings⁵⁴. In an assessment of *Arabidopsis* plants exposed to the proteasomal inhibitor Syringolin A, investigators monitored protein abundance as well as characterizing 1791 ubiquitinated proteins. More proteins decreased than increased in abundance in the treated tissues, suggesting that besides degradation via the UPS, transcriptional and post-transcriptional processes were also altered⁵⁵. Finally, the small ubiquitin-like modifier (SUMO) has been shown to slow degradation of pre-existing protein conjugates in heat-stressed *Arabidopsis* seedlings, and may indirectly affect protein synthesis through transcriptional alterations⁵⁶.

A plethora of non-enzymatic modifications to proteins can occur during oxidative stress, such as nitrosylation and carbonylation. These modifications can result in changes in protein structure and unfolding, exposing hydrophobic residues to the cellular environment, which can serve as a signal for degradation. These modifications can function in signaling pathways, with S-nitrosylation of cysteine residues being one possible example⁵⁷. Some modifications, such as the formation of disulfide bridges and methionine oxidation, are reversible; other modifications, such as carbonylation, are not. Extensive oxidation, like other abiotic stresses, may lead to the formation of protein aggregates, as is the case for Rubisco activase and catalases, which might prove fatal for the cell due to the loss of protein function, in the case of these two proteins, this would entail a reduction in photosynthetic capacity and oxidative protection, respectively⁵⁸. In some cases, post-translational modification resulting from oxidation may lead to the removal of damaged proteins from plant cells⁵⁹. A more complete discussion of post-translational modifications and their role in protein function and stability in plants is provided elsewhere⁶⁰. A key uncertainty is how and why some proteins are degraded so quickly and whether this relates to their modification post translation or association with other rapidly degraded proteins. In some cases the need for rapid degradation is clear. The iconic example being the D1 protein of photosystem II, which is damaged by reactive oxygen species⁵². Similarly, a short half-life would allow a regulatory protein to rapidly respond to a changing environment; hormone response factors⁶¹ and hormone induced transcription factors⁶² are examples of this. In other case the need for rapid turnover is less apparent.

Interplay between protein turnover and metabolism

Understanding the relationship between protein turnover and metabolism in plants could pave the way to engineering crops for better yields in a changing environment. As an example, consider the role of D1 protein in photosystem II in photosynthesis and photoinhibition. The D1 protein is damaged as a result of protein function and when not repaired results in a reduction of the conversion of light to chemical

energy, i.e. photoinhibition^{52,63}. Plants have evolved repair systems to minimize this downtime, including removal of the damaged D1 subunit, degradation via compartment localized-proteases, and replacement with a newly synthesized D1 molecule. However, when the plant is exposed to high light the D1 protein cannot be replaced rapidly enough and photoinhibition can occur. As a result, carbon assimilation is reduced and growth is limited. When high light conditions are combined with other abiotic stresses like drought or cold, the photosystem II repair system is damaged by reactive oxygen species, further reducing photoassimilation. However, researchers have shown that it is possible to mitigate some of the negative effects of combined stresses on the D1 protein, at least in tobacco, through the introduction of a bacterial catalase gene, relieving some of the oxidative stress⁶³.

The co-regulation of plant protein turnover and metabolism helps to ensure the conservation of cellular energy. For instance, during darkness protein synthesis - one of the more expensive metabolic processes - is slowed so as to ration out available energy reserve³. During these periods autophagy helps to remobilize resources and to meet additional energy needs²³. Mutants unable to engage in starch synthesis or autophagy have dramatically reduced growth rates under normal plant growth conditions and catabolic pathways are activated²³.

In other instances plants have evolved strategies with regards to protein turnover and metabolism that would seem to put the plants at a disadvantage, at least from the perspective of crop yields. When plants experience prolonged periods of abiotic stresses such as drought and salinity, premature senescence occurs and photosystem proteins and chlorophyll are degraded, compromising seed production and reducing final yields^{64,65}. Alternatively, some stay-green mutants do not suffer this effect, and chlorophyll and the associated photosystems remain intact and functional, and therefore contribute to final yield⁶⁵. This reduction in the catabolism of photosystem components and chlorophyll in the stay-green mutants enhances yield under stress relative to non-mutant plants⁶⁴. Senescence is known to be induced by ethylene, which is made via the 1-aminocyclopropane-1-carboxylic acid synthetic pathway. Stay-green plants that block ethylene production via this pathway, delaying senescence and thereby reducing the rate of degradation of photosystem proteins and chlorophyll and enhancing production, are being engineered⁶⁶.

The role of development and environmental change

Many of the protein turnover studies conducted to date in mammalian, yeast, and plant systems have been carried out in steady-state conditions, that is, when the rate of protein synthesis and degradation remain constant^{5,12,32-34,36,46,61}. There are periods in plant development during vegetative growth when the assumption of steady state protein dynamics is reasonable. However, the proteome of plants continually changes (Figure 2) in response to shifts in the environment, be it the daily cycle in light and temperature, or the imposition of abiotic stresses such as nutrient deprivation, salinity, and drought (Figure 3). Under these circumstances, k_s and/or k_d will change for specific sets of proteins. The largest changes in synthesis and/or degradation are likely to occur during the early stages of these new conditions, when the cell is finding a new steady state. Many proteins are also expected to display non-steady state dynamics during developmental transitions (Figure 2).

The measurement of protein synthesis and degradation under non-steady state scenarios is challenging because of changes in protein abundance^{11,35}. However, measurements of k_s and k_d have been made in plant systems under dynamic conditions. Using stable isotopes as a tool for measuring k_s and k_d , the first proteomic study in plants to measure protein turnover in a non-steady state scenario assessed rates of synthesis and degradation in *Arabidopsis* cell culture; as these cultures age and develop cell

density increases and resources are depleted³¹. In general proteins involved in protein synthesis and degradation, as well as RNA/DNA binding proteins, were degraded most rapidly. In addition, selective translation, complemented by tight control of protein degradation, was shown to play an important role in determining protein abundance during germination in *Arabidopsis*²⁶. Similarly, selective translation was shown to play a more important role than transcription in regulating changes in protein abundance in *Arabidopsis* on prolonged exposure to high light conditions¹⁷.

Aside from the direct measurement of protein synthesis and degradation, much can be gleaned about developmental changes in protein turnover from studies of transcript and protein abundance. In particular, there is a rich literature associated with leaf development in maize. For instance, a transcript-level study of leaf development and cell type found that approximately two thirds of all transcripts varied with the developmental stage, while one in five transcripts varied between bundle sheath and mesophyll cells⁸. Proteomics has also been applied to describe developmental differences in maize leaves^{15,40}. In one of these studies, label-free proteomics was integrated with quantitative microscopy techniques to analyze changes in the abundance of over 2,600 proteins during leaf development in maize; the data were to assess organelle biogenesis, protein co-expression patterns and several other aspects of development¹⁵. Interestingly, mesophyll and bundle sheath cell differentiation were found to take place around the time of the source/sink transition for assimilated carbon. There is also an extensive knowledge base for rice, again representing opportunities for future exploration. For example, proteomic studies have explored the phenomenon of heterosis in rice; a comparison of hybrids to parent lines during development revealed that proteins involved in photosynthesis, glycolysis, and defense were elevated in the hybrid plants⁶⁷. In another report investigators studied the dynamics of seed filling under different temperature regimes⁶⁸, and in other studies changes associated with germination under variable conditions^{69,70}.

Proteomic studies are reinforcing what earlier studies suggested - that in steady and non-steady state scenarios there is a need for symmetry in responses, both in terms of a balance between protein generation and destruction as well as energy intake and consumption. The abundance of most proteins does not need to change for day to day operations of the cell when conditions change, so that coordinated changes between synthesis and degradation can maintain this balance. As an extension of this symmetry, when plants are starved or grown under low nitrogen conditions, protein degradation is increased in the older parts of the plant, in order to generate respiratory substrates and mobilize resources for synthesis in developing tissues²³. As a counterpoint, in other stress scenarios protein degradation is known to decrease either due to misfolding of proteins and overloading of the UPS system⁷¹, or through an elegant mechanism by which the ubiquitin ligases, PUB22 and PUB23, target regulatory proteins in the proteasome lid, thereby destabilizing it and decreasing proteasomal activity⁷¹. Translation is known to decrease during extended darkness as well as other stressors³.

Protein dynamics in complex real-life scenarios

Most of the studies discussed have been conducted in laboratory environments, under relatively sterile conditions. However, in the real world plants don't germinate on agar plates or grow in hydroponic media or sterilized soil. Rather, plants germinate and grow in soils which are complex biomes composed of other plants, bacteria and fungi (Figure 2). This can give rise to biotic stresses, including herbivory, and pathogen and parasite attack, but may also result in symbiotic relationships. We are only beginning to appreciate this complexity and to address these issues experimentally at the level of the proteome. Many investigators have focused on the response of plants to the symbiosis with arbuscular mycorrhizal fungi and rhizobial bacteria, because they enhance plant nutrient uptake and nitrogen fixation, respectively. In

an effort to better understand initiation of the symbiosis between the legume *Medicago trunculata* and symbiont nitrogen fixing bacteria, researchers quantified thousands of proteins and phosphosites following exposure of Medicago roots to rhizobial nodulation factors. A number of specific changes were evident at the protein level, which could be associated with changes in synthesis or degradation. Significant changes were also apparent in the phosphosites of several proteins that are likely involved in signaling cascades¹⁴. In a longer term study of over 1,200 membrane proteins in Medicago, 96 were enriched after long term growth with mycorrhizal fungi⁷². In another study of Medicago, investigators attempted to decipher the relationship between host and symbiont under abiotic stress by studying nitrogen fixation in a Medicago/nitrogen-fixing bacterial growth experiment under drought stress, when nitrogen fixation is reduced. Despite no apparent sulfur limitation, methionine and ethylene biosynthesis pathways were down-regulated⁷³. As a final example of increasingly challenging experiments, investigators conducted a meta-proteomic analysis of bacteria associated with field-grown rice plants that appeared to have roots that were nitrogen fixing and methane consuming. By combining proteomic data, where thousands of bacterial proteins were characterized, with catalyzed reporter deposition-fluorescence in situ hybridization assays, a species of *Methylosinus* spp. was identified as the symbiont likely to be catabolizing methane while also fixing nitrogen for the rice⁷⁴. None of these studies directly addresses the issue of whether the measured protein abundances were the result of changes in the rates of protein synthesis and/or degradation. However these complex designs that either mimic or monitor agronomic scenarios are amenable to isotope labelling strategies to study the exchange of nutrients and metabolic products (Figure 3), as well as the impact of the symbiosis on proteome turnover in both host and symbiont (Figure 2).

Balance, regulators and agronomic gain

It is clear that plant protein synthesis and degradation are highly coordinated processes that are balanced in steady as well as non-steady conditions, with reduced degradation when synthesis slows and the inverse when synthesis speeds up, as is the case during development and environmental change. Experimental tools are now available to quantify the contribution that transcriptional, post-transcriptional, translational, and post-translational changes make to these outcomes. Additionally, regulators such as mTOR and effectors like the E3 ligases that provide specificity for this balance under different physiological scenarios are beginning to be described. The next step is to characterize the rest of the regulators, effectors, and targets of changes in protein synthesis and degradation under conditions of interest, in order to monitor not just gene expression but the downstream cost and effectiveness of different strategies to alter steady-state protein abundances. With this information we can then attempt to change and improve these systems for agronomic gain.

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CJN and AHM co-wrote and edited the review and generated the figures.

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There are no competing financial interests of the authors.

Figure Legends

Figure 1. Plant protein synthesis and degradation machinery and its regulation. Within the plant cell, protein synthesis is carried out in the cytosol by the action of 80S ribosomes while in mitochondrion and plastids, proteins are synthesized by bacterial-like 70S ribosomes. Besides transcript abundance, other factors control protein synthesis, including the secondary structure of mRNA and diurnal factors such the light-regulation of plastid protein synthesis. The ubiquitin-proteasome system (UPS), which uses a series (E1, E2, and E3) of conjugating enzymes to label proteins for catabolism with much of the specificity provided by the large number of E3 ligases. For example, the SP1 protein localized in the outer plastid membrane and facing the cytosol is a RING-type E3 ligase that interacts with the Translocon of the Outer Chloroplast (TOC) import machinery and UPS, controlling abundance of specific subunits and thereby regulation of transition between plastid types and life stages¹⁸. Additionally, UBP27, an ubiquitin protease, is located on the outer mitochondrial membrane and is involved in mitochondrial morphology²². There are also specific protease that degrade plastid and mitochondrial proteomes, notably the AAA-class Lon, FtsH and Clp proteases^{7,24,25,75,76}. Post-translational modification processes can influence degradation, for example DELLA proteins regulate gibberellin-dependent growth processes in plants and their degradation rate is controlled by phosphorylation status, with catabolism ultimately mediated by another E3 ligase, SCF⁶. In addition, phosphorylation of the D1 and D2 proteins of PS II affects their stability⁵². Autophagy, another path for protein degradation, occurs in the vacuole and can play an important role under various physiological scenarios in plants²³. A key regulator in these processes is the mitogenic target of rapamycin (mTOR), which inhibits autophagy and stimulates protein synthesis via its effects on small ribosomal subunit 6 kinase (S6K) and the E2Fa transcription factor¹⁰.

Figure 2. Proteome development and steady-states during the life-cycle of plants. When plants germinate, they degrade storage proteins for energy and to make building blocks for the proteins that need to be synthesized for the later stages of plant development. In aerial tissues the machinery needed

for cell division, expansion and primary metabolism comprise a large part of this initial investment. Shortly after seedling formation, de-etiolation results in a large expenditure on photosynthetic proteins and over time investment in defense and secondary metabolism proteins increases. Finally, as leaves senesce and fruiting begins, plants degrade the photosynthetic components and other housekeeping proteins in order to provide the energy, amino acids, and other building blocks for storage proteins. This developmental process results in periods of apparent steady-state, where synthesis and degradation are balanced and plant tissues grow through cell division and expansion. Development also produces periods of rapid change where transitions result in major re-structuring of the proteome through a combination of alterations in synthesis and degradation. Analysis of proteome dynamics in plants requires a close coordination of experiments with this development pattern of plants. In root tissue, the early establishment of protein machinery for macro- and micronutrients uptake is essential for plant growth. Development of symbioses and nodulation with soil bacteria (blue) in some plant species allows nitrogen fixation. Establishment of mutualistic interactions between arbuscular mycorrhizal fungi (magenta) allows the exchange of carbon from plants to fungi for enhanced uptake of nutrients such as phosphate. Exchange of nutrients as protein building blocks means that root and fungal/bacterial biome proteomes are linked and the degradation and synthesis of proteins in these systems should be considered together as they respond to the environmental and developmental cues from both plant and biome.

Figure 3. Combining quantitative protein synthesis and degradation measurements with physiological studies. Left - using stable-isotopic labels ($^{13}\text{CO}_2$, $^{15}\text{NH}_4$, $^{15}\text{NO}_3$, H_2^{18}O , $^2\text{H}_2\text{O}$), isotopic tracers can be incorporated into amino acids, the building blocks for proteins. Plant samples of interest can then be fractionated into cell types, subcellular compartments, and protein complexes using various separation techniques. Using MS-based proteomics for peptides from these samples, the decay of peptide populations derived from proteolysis of pre-existing and newly synthesized proteins can be monitored simultaneously allowing calculation of k_s and k_d and interpretation of steady-state and non-steady state experiments. Right - these techniques can be combined with physiological measurements such as gas exchange and water and nutrient transport that show variation in response to environmental cues in order to determine how various physiological parameters interact. By integration of these different methodologies with protein turnover techniques, we can begin to estimate costs of protein synthesis and degradation for various plant tissues and cell types under various biological scenarios. By taking into account both developmental and environmental processes using new and more established techniques, we can accelerate our ability to model plant physiology and use these tools in a predictive manner for rational engineering of plants.

Isotope incorporation measurements

Physiological rate measurements

fractionation:
tissue
cell type
subcellular
affinity purification

MS analysis:

Calculation
of K_s & K_d

steady-state:

define fast and
slow turnover proteins

linked turnover rate to
function and regulation
within pathways
and complexes

non-steady state:

define proteins changing
in degradation or
synthesis rate

map timing of changes
to alteration in protein
abundance

-Measure absolute costs of synthesis & degradation
-Integrate carbon partitioning
with energy budgets
-Correlate proteome dynamics with
physiological measurements
-More holistic understanding of plant responses
for modeling plant function