An examination of Job’s rule: protection and repair of the proteins of the translational apparatus in seeds

Lynnette M.A. Dirk and A. Bruce Downie

Department of Horticulture, Plant Science Building, 1405 Veterans Drive, University of Kentucky, Lexington, KY 40546-0312, USA and UK Seed Biology Group

Abstract
The proteins produced just prior to maturation desiccation in the developing, orthodox seed, are stored in the desiccated state and recruited as the functional proteome upon imbibition. For the resumption of protein function, these stored proteins must be protected from permanent denaturation while dehydrating, throughout desiccation, and during rehydration. For some forms of damage there is the possibility of repair following imbibition potentially coordinated with de-aggregation into monodispersed polypeptides capable of refolding into a functional configuration. While studying aspects of the natural protection and repair mechanism in seeds, evidence has accrued that those proteins directly involved in translation are particular targets of both protection and protein repair. Such a phenomenon was first described by Rajjou et al. (2008) examining the frequency with which proteins involved in translation were identified as differentially abundant between aged and un-aged Arabidopsis seeds and the translational competence of aged versus un-aged seeds. The inference drawn from these observations was that, of all the stored proteins, it is imperative that those involved in translation endure desiccation, quiescence and rehydration in a functional state if the seed is to survive. Proteins involved in any other process other than translation can be replaced from the stored transcriptome or by de novo transcription but no mRNA is of value without the translational machinery. This has become known as ‘Job’s rule’ in honour of the laboratory from which this hypothesis was first put forward (Rajjou et al., 2008). We review in this manuscript the evidence accrued to date on which Job’s rule is based.

Introduction
Water is essential for life and yet there are organisms that have phases of their life cycle during which they can withstand dehydration to 10% water content on a dry weight basis (0.1 g H2O g⁻¹ dry mass; Alpert, 2005). This phenomenon, known as ‘anhydrobiosis’ or ‘life without water’ (actually with little water), is an attribute of many bacteria (Mattiimore and Battista, 1996; Billi and Potts, 2002), fungi (Mtwisha et al., 1998), lichens (Kranmer et al., 2005), and some animals (Browne et al., 2002; Tunnacliffe and Lapinski, 2003; Browne et al., 2004; Hengherr et al., 2008; Menze et al., 2009). In the kingdom Plantae, certain algae and mosses exhibit vegetative anhydrobiosis (Clegg, 2001; Oliver et al., 2000) as do some ferns (Stuart, 1968; Muslim and Homann, 1992) and those higher plant species constituting the ‘resurrection plants’ (Moore et al., 2009; Costa et al., 2017). The result of sexual reproduction for many of the gymno- and angiosperm species worldwide are orthodox seeds (Roberts, 1973) which are capable of desiccation. Largely due to the capacity to dehydrate, these seeds remain viable in extremes of temperature (Ellis et al., 1988; Vertucci, 1989) and, in some instances, beyond a millennium (Shen-Miller et al., 1995; Sallon et al., 2004), or even unprotected in space (Tepfer et al., 2012). A continuing, fascinating quandary is how this anhydrobiosis, leading to such resilience, is possible, prompting attempts to gain insight into the molecular mechanisms underlying the attribute (Potts et al., 2005; Nambara and Nonogaki, 2012). On a practical level, understanding the components of anhydrobiosis would lead to various uses in plasma- and pharmaceutical-preservation at ambient temperatures, reductions in transportation costs, and long-term stasis of complex cell assemblages without freezing.

Anhydrobiosis and the natural protection and repair mechanism
Orthodox seeds (Roberts, 1973) must weather a host of detrimental events when becoming, or while, dehydrated allowing the vigour of the seed, and the seedlings established from them, to remain uncompromised (Fig. 1). The natural protection and repair mechanism (Fernandez-Marin et al., 2013) is a vital but understudied facet potentiating desiccation tolerance and is linked to seed/seedling vigour by preventing or counteracting damage sustained...
while entering or during anhydrobiosis. It is, consequently, the foundation of crop field performance (Li et al., 2017b). The fundamental importance of the orthodox seed as the cornerstone of agriculture (Li and Pritchard, 2009) is in stark contrast to our lack of a basic understanding of the molecular mechanism of action for many presumptive protective molecules in shielding the cellular milieu from dehydration stress (Battaglia et al., 2008; ElSayed et al., 2014; Kester et al., 1997; Van den Ende, 2013). Important for anhydrobiosis and the focus of research activities worldwide (Berjak, 2006), protective mechanisms include reduction of reactive molecular species (Bailly, 2004) particularly in the prevention of lipid peroxidation (Debeaujon et al., 2000; Sattler et al., 2004) and vitrification of the cytoplasm upon water removal (Buitink et al., 1998, 2000; Sun and Leopold, 1997; Wolkers et al., 1998a,b). The cytoplasmic phase transitions from liquid-to-viscous-to-glass, are thought to increasingly impede deleterious biochemical reactions while progressively dampening respiration (Leprince et al., 2000). Those cellular components, dependent on water to maintain their structure/function, are thought to be protected using so-called ‘water replacement’ by specific, non-reducing oligosaccharides (Crowe et al., 1998; Clerkx et al., 2004; Li et al., 2017b). It is thought that, in conjunction with highly hydrophilic proteins, these oligosaccharides can also enhance the quality and persistence of the glassy state.
widely held tenet is that (Fig. 1). Using Cordycephin, Bai for the completion of seed germination, at least in Arabidopsis (Isbell and Frush, 1958), in addition to the capacity of oligosaccharides to form a glass. Additional protection is thought to be mediated by intrinsically disordered proteins, abundant late during embryogenesis, through the prevention of aggregation of cellular constituents as water is withdrawn and the distance between macromolecules diminishes (Goyal et al., 2005; Boucher et al., 2010; Chakrabortee et al., 2012).

Some cellular constituents suffering damage while dehydrated can be repaired upon rehydration. These repairs include recognition, excision and ligation of damaged bases through apurinic or apyrimidinic intermediates (Dendoy et al., 1987) and ligation of outright DNA strand breaks (Huang et al., 2008; Waterworth et al., 2010). Many proteins partially damaged or denatured during desiccation or upon rehydration, can be retrieved from aggregation (Boucher et al., 2010), refolded (Tonsor et al., 2008), and repaired (Grimaud et al., 2001; Holmgren et al., 2005; Smyczynski et al., 2006; Oge et al., 2008; Nayak et al., 2013; Verma et al., 2013).

Ten years ago, while comparing proteomic alterations in Arabidopsis thaliana seeds during natural and artificial ageing (controlled deterioration), Rajiou et al. (2008) postulated that the proteins involved in translation, although desiccated, must be maintained during quiescence in a functional state for the maintenance of seed vigour. They reasoned that if sufficient numbers of seed cells lose translational competence, neither the stored – nor de novo – synthesized transcriptome (Nakabayashi et al., 2005) can rescue translational capability and the seed will die (Fig. 2). This postulate (named Job’s rule in honour of Dominique Job from whose laboratory this concept arose) has been supported in intervening years when proteins involved in translation have been shown to be among the preferred client proteins of presumptive desiccation protective mechanisms as well as particular targets of protein repair processes. This paper will present the existing documentation for Job’s rule acquired by examining the proteins identified as preferred client proteins for protection and repair mechanisms.

**Job’s rule:** The proteins critical for translation must endure desiccation, quiescence, and rehydration in a functional state if the seed is to survive to complete germination.

**Death by other means**

Maintaining the translational apparatus functional is not a panacea preventing seed death. The variety of cellular components that must be protected from extensive dysfunction so that organisms might survive desiccation are considerable and the list of issues impacting survival of desiccation continues to grow as our sophistication concerning the variety of tissues, organelles and molecules involved in longevity increases (Cheah and Osborne, 1978; Oliver et al., 2005; Potts et al., 2005; Berjak, 2006; Rebecchi et al., 2007; Moore et al., 2009; Sano et al., 2016; Costa et al., 2017; Pereira Lima et al., 2017). An example of a recent overturn of a previously held tenet is that de novo transcription appears to be crucial for the completion of seed germination, at least in Arabidopsis (Fig. 1). Using Cordycephin, Bai et al. (2018) determined that blocking transcription reduced the completion of seed germination to near zero. At least in Arabidopsis, the capacity for de novo transcription, divorced from the maintenance of an intact genome (i.e. DNA integrity in these studies was unaffected and therefore, not the cause of poor transcription), must be safeguarded to maintain seed longevity. The integrity of the genome in the dehydrated state is critical to seed longevity (Waterworth et al., 2015) as is the integrity of the stored transcriptome (Fleming et al., 2017) (Fig. 1). The protection of membranes from peroxidation both drastically and positively impacts seed viability (Sattler et al., 2004; Mene-Saffrané et al., 2010; Xu et al., 2015). Even the proper size and integrity of oilbodies is crucial for the survival of desiccated soybean cotyledons (Schmidt and Herman, 2008).

The above pathways, the dysfunction of which can lead to seed death, places the fate of the stored proteome, and its influence on seed longevity (the focus of Job’s rule), in context. The proteome is only one subset of a larger assemblage of molecular entities that must retain biological function if the seed is to survive. Among the proteins comprising the stored proteome, those directly and critically involved in translation occupy a special status and must be protected from, or repaired after, dysfunction if the seed is to survive.

**What proteins encompass those ‘critical for translation’?**

It is imperative to realize that the focus of Job’s rule on ‘proteins critical for translation’ includes all proteins that are indispensable for production of active proteins from mRNA. But what are these? An attempt at defining a Minimal Protein Synthesis Machinery (MPSM) has been undertaken in some prokaryotes (Grosjean et al., 2014). Pared down, this assemblage still incorporates 104 core proteins with an additional 25 persistent proteins in seven different protein categories: (1) ribosomal proteins; (2) rRNA modification; (3) ribosome assembly and protein maturation; (4) RNA processing; (5) tRNA modification; (6) tRNA aminocacylation; and (7) translation factors (Grosjean et al., 2014). In eukaryotes this list may be expanded to include an eighth category of proteins responsible for transporting nuclear encoded proteins critical for translation into the organelles. Using this list as a guide, we sought evidence that proteins in these categories were inherently resilient or preferential client proteins of the natural protection and repair mechanism to provide support for Job’s rule. Where no evidence has been published for a particular category, we hope that this review will spur future research efforts in this direction. Rather than attempt to list evidence under these eight categories, we have found it to be less repetitive to mention the Category when a report has been published pertaining to its resilience, protection or repair.

Proteins involved in translation that are not associated with Job’s rule include those that peripherally influence or modulate translation but are not critical for it. These may include the proteins of the TOR protein complex (e.g. TARGET OF RAPAMYCIN; TOR and conserved TOR associated proteins; REGULATORY ASSOCIATED PROTEIN OF TOR 1B (RAPTOR1B) and LETHAL WITH SEC-THIRTEEN PROTEIN 8 (LST8)) the complex of which is known to influence the efficiency of translation but is not critical for it (Kravchenko et al., 2015). The WD40-repeat protein GIGANTUS1 (GTS1) is also capable of altering ribosomal morphology, is co-expressed with several r-proteins, and is predicted to interact with at least two r-proteins, potentially influencing translation, but GTS1 is deemed non-essential for translation (Gachomo et al., 2014). One can imagine that
plasmodesmatal proteins, crucial to protein trafficking throughout discrete, symplastically continuous cell assemblages in the mature embryo (Kim et al., 2002; Stadler et al., 2005), may revitalize cells in which translational competence has declined below a critical threshold that the surrounding cells can complement by sharing translational components, predicated on mechanisms to facilitate passage through the size exclusion limit of their plasmodesmata. These plasmodesmatal proteins are, however, not directly involved in translation and are, therefore, excluded from Job’s rule.

Support for Job’s rule

The proteins of the translational apparatus possess a remarkable resiliency

In a survey of protein half-lives in barley leaves, Nelson et al. (2014) found those proteins associated with the translational apparatus (no specific category) to be particularly long-lived, suggesting that these proteins might somehow be inherently resilient or that they are a class of the proteome to which partiality is
shown by the protective and/or repair mechanisms of the cell. Indeed, the ribosomal proteins (r-proteins; Category 1) from diverse species, once assembled into ribosomes, are particularly long-lived, at least in the cytoplasm (Boisvert et al., 2012; Christiano et al., 2014; Li et al., 2017a).

The r-proteins of the large- and small-ribosomal subunits are intimately involved in translation. Some insight into the long-lived nature of some of the r-proteins has been linked to their high positive net charge at physiological pH, with some species’ large subunit r-proteins all possessing a net charge at physiological pH greater than 7 (Fedyukina et al., 2014). This has relevance for protein and ribosomal stability because Lawrence et al. (2007) demonstrated a surprising capacity of protein variants, engineered to impart to them either a positive or a negative ‘supercharge’, to re-gain structure and function following stressful events (in this case supra-optimal temperatures). They demonstrated that supercharging prevents protein aggregation upon denaturation and yet does not dramatically inhibit subsequent refolding into an active protein. Inherent r-protein positive supercharging is not strictly due to functional demands on proteins that must associate with negatively charged r-, t- and mRNA. Intriguingly, Fedyukina et al. (2014) have recorded a surprising plasticity in the net charge of r-proteins of the large subunit with those from halophiles considerably less positively charged than those from non-halophilic organisms. Upon further investigation, it was evident that r-proteins of the large subunit are generally segregated into positively and negatively charged protein moieties, a characteristic that becomes more pronounced in the halophile. The halophile negatively charged amino acids were found to be placed in solvent exposed positions, potentially better competing with salt for water for protein hydration while the positively charged portions of the protein tended to be buried within the protein-ribosomal RNA core (Fedyukina et al., 2014). In keeping with Job’s rule, such durability is a useful attribute if the proteins comprising the translational apparatus must remain in an active form throughout quiescence and are of such vital importance to the longevity of seeds following imbibition.

In some organisms, including plants, ribosomal protein (r-protein) families have numerous paralogous members (Barakat et al., 2001; Carroll et al., 2008; Carroll, 2013; Hummel et al., 2015). One hypothesis concerning this r-protein diversity is that the r-protein paralogues can impart to the translational machinery selectivity regarding which mRNAs are preferentially translated (Hummel et al., 2015), constituting a so-called ‘ribosome filter’ (Mauro and Edelman, 2002). Indeed, such are the subtleties of r-protein paralogue alterations to the ribosome, influencing the preference of the ribosome for translating specific mRNAs, that, in yeast, paralogues of the LARGE SUBUNIT RIBOSOMAL PROTEIN1 (RPL1; a or b), although identical in amino acid sequence, somehow dramatically bias the mRNA species translated by the ribosomes containing one or the other RPL1 paralogue (Segev and Gerst, 2018). Despite this redundancy, many genes encoding r-proteins belong to cytoplasmic, mitochondrial (Tzafir et al., 2004; Zhang et al., 2015; Robles and Quesada, 2017), as well as plastidial (Tsugeki et al., 1996; Gong et al., 2013) ribosomes are known to be lethal when mutated (Lloyd and Meinke, 2012). As our sophistication regarding translation during germination increases, the identities of r-protein paralogues, and their post-translational modifications (Carroll, 2013; Sanchez-de-Jimenez et al., 1997), that are crucial to selective translation at various stages of germination, may be revealed to be absolutely required for the recovery from quiescence and the completion of seed germination (Galland et al., 2014; Babouss-Serhal et al., 2015; Galland and Rajou, 2015; Bai et al., 2017). In this vein, it should be noted that some of the first transcripts to be translated following imbibition, are those in the stored transcriptome encoding ribosomal proteins (Beltran-Pena et al., 1995; Tatematsu et al., 2008; Weitbrecht et al., 2011) prioritizing the replacement of critical translational capacity with what might be the last functional translation of which the rehydrated, aged ribosome is capable! This is entirely consistent with the tenets of Job’s rule.

The r-proteins are only one of seven different categories listed as crucial for translation (Grosjean et al., 2014). In studies of aged seeds, certain members of Categories 3 and 7, (translation factors; initiation and elongation factors, chaperonins) have been documented to decline in abundance as ageing progresses (Min et al., 2017; Wang et al., 2012). The distinction to be made here is that the studies indicating that Category 3 and 7 proteins declined in abundance were conducted using hydrated seeds exposed to high temperatures (accelerated ageing conditions) which may or may not reflect the dynamics of protein destabilization under natural ageing conditions (Schwember and Bradford, 2010).

Protection: Proteins involved in translation may be protected from oxidation in the quiescent seed

Oxidation is a stress assailing the components of cells of the seed to which lipids, nucleic acids and proteins are all susceptible (El-Maarouf-Bouteau et al., 2013). The reactive oxygen species (ROS) are also potent signalling molecules. Hence the hydrated cell must permit sufficient ROS generation to fulfil relevant signalling while dampening ROS quantities below a threshold where cellular constituents are damaged by them (Baillie et al., 2008) and the hydrated cell employs a plethora of mechanisms to establish and maintain redox balance (Apel and Hirt, 2004). The sulfur-containing amino acids (AAs; cysteine and methionine) are prone to oxidation (Levine et al., 2000). Both of these oxidations, proceeding no further than cysteine sulfenic acid or methionine sulfoxide, respectively, are repairable by enzymatic reduction (Tarrago et al., 2009; Meyer et al., 2012; Akter et al., 2015; Waszczak et al., 2015) and even cysteine sulfenic acid is reversible in some cellular compartments (Rey et al., 2007). Other AAs (proline, histidine, lysine, arginine, tyrosine and tryptophan) are also subject to oxidation but, to date, no reductive repair mechanism for these has been identified (Rinalducci et al., 2008; Sweetlove and Moller, 2009). Some proteins in cell-free extracts from desiccated seeds appear to be preferentially oxidized; this damage occurring to these specific proteins at frequencies far surpasses their proportionate abundance in the cell (Oracz et al., 2007; El-Maarouf-Bouteau et al., 2013). This oxidation is presumed to have occurred during the sojourn of the seed in the dehydrated state (Gao et al., 2013) although it is impossible to emphatically state that the oxidation does not take place immediately upon addition of aqueous buffer used to extract the proteins from the desiccated seed, an ubiquitous caveat dogging most assays of dehydrated tissues. Nevertheless, in support of Job’s rule, of those proteins identified as preferentially oxidized from desiccated seed, only one is involved in translation (eukaryotic ELONGATION FACTOR2 [eEF2]; Category 7; Job et al., 2005; Oracz et al., 2007) and its oxidation in dry seeds is associated with dormancy alleviation in Helianthus annuus L. (sunflower), stimulating the completion of germination upon Category 1
subsequent hydration, constituting a time-dependent signal eliciting a specific event (dormancy alleviation), rather than random protein damage.

In contrast, hydration of the seeds prior to protein extraction results in the oxidation of many proteins directly involved in translation (Job et al., 2005) so these translation-associated proteins, present in the desiccated cells of the seed, are not impervious to oxidation but seem, rather, to be specifically protected from it while dehydrated. The means by which the proteins that are prone to oxidation are rendered susceptible to it remains relatively unknown, as does the means by which proteins involved in translation are seemingly protected from oxidation while desiccated. One intriguing example of the former is the use, in animal mitochondria, of AA substitution through alternative codon translation, resulting in methionine (or N-formylmethionine) residues for both codons AUG and AUA (usually isoleucine). In this instance, solvent-exposed Met residues are thought to act as oxidation decoys, sacrificing their integrity to protect other mitochondrial constituents from ROS while, due to their peripheral placement in the protein they act like a ROS sponge, minimally influencing the ROS sponge protein’s structural integrity and hence, function (Bender et al., 2008). A repair pathway capable of reducing methionine sulfoxide also exists in the cell, which can render this damage temporary (Achilli et al., 2015). The seed storage proteins (one class of seed stored proteins) which are degraded to supply energy, nitrogen and carbon for the establishing seedling, are prone to all manner of damage during quiescence. Oxidative alterations have been suggested to be evidence of a role for the abundant storage proteins (Nguyen et al., 2015) in seeds to soak up ROS, reducing the titre of the damaging ROS during quiescence. An alternative opinion is that the storage proteins, destined for degradation and present in a compartment bereft of some repair mechanisms, are simply unworthy of repair (Dinkins et al., 2008), slated as they are for destruction. There is even a role for phytic acid (frequently associated with seed storage proteins as inclusion bodies) as an anti-oxidant contributing to the seed longevity of maize (Zea mays) seeds (Doria et al., 2009).

Protection: LEA protein association with proteins involved in translation

The LATE EMBRYOGENESIS ABUNDANT proteins (LEA proteins) are thought to protect intracellular membranes and proteins of seeds while drying and during their sojourn in the desiccated state (Hundertmark et al., 2011). LEA proteins have been suggested to act as molecular spacers in the increasingly crowded intracellular milieu during dehydration (Goyal et al., 2005) and some have been shown to possess remarkable anti-aggregation properties (Boucher et al., 2010). Some of the LEA proteins have been demonstrated to exert a protective function for membranes of particular composition (Thalhammer et al., 2010; Tolletter et al., 2010; Eriksson et al., 2011; Thalhammer et al., 2014; Eriksson et al., 2016) and some to stabilize sugar glasses (Wolkers et al., 2001; Shimizu et al., 2010). Certainly the cosmopolitan distribution of usually two or more members of the LEA proteins within compartments of the plant cell (Candat et al., 2014) augurs well for a general, shielding mechanism, redundantly backed up (Chakrabortee et al., 2012). Recently, this view is being altered due to the recovery of a diversity of specific phenotypes upon mutation of a single LEA (Manfre et al., 2006; Chen et al., 2010; Olvera-Carrillo et al., 2010; Salleh et al., 2012), scarcely possible if LEAs all acted as general shield molecules with multiple members present in cellular compartments. While demonstrations of certain of the LEA proteins safeguarding, to some degree, the function of commercial enzyme preparations abound (e.g. Hara et al., 2004; Reyes et al., 2005; Kovacs et al., 2008; Liu et al., 2010; Zhang et al., 2014), efforts to identify particular endogenous proteins that are bound by explicit, conspecific LEA proteins have also been successful (Xie et al., 2012; Rivera-Najera et al., 2014; Zhang et al., 2014; Hernandez-Sanchez et al., 2017). Consistent with the tenets of Job’s rule, preferred client proteins bound by a SEED MATURATION PROTEIN (SMP) family Arabidopsis LEA protein, and by its soybean orthologue, belonged to Categories 1, 4 and 7 of translation (Kushwaha et al., 2012) providing evidence that at least some of the LEA proteins can bind (and presumably protect) proteins of the translational apparatus (Kushwaha et al., 2013). Loss of function of the Arabidopsis SMP LEA, while not lethal to the seeds, does remove the capacity of smp1 seeds to enter thermodormancy in response to thermal insult applied during germination (Chen et al., 2010), seemingly negating the seed hydration memory of the stress (Fig. 1).

The involvement of the LEA protein SMP1 in binding (presumably protecting) proteins involved in translation may be used as a paradigm to suggest that certain LEA proteins may physically shelter oxidation-sensitive AAs of their client proteins from this modification in the quiescent seed. Certainly the region of the client proteins to which both Arabidopsis and soybean orthologues of SMP1 bound was consistently the most, or among the most, hydrophilic of the client proteins (Kushwaha et al., 2013), suggesting that these regions are solvent exposed and therefore would be the most susceptible to oxidative damage. Such physical protection from oxidation has been demonstrated for the subunit proteins comprising the 12S cruciferin storage proteins where the β-subunits, thought to be buried within the α-subunits, are 6-fold less oxidized in the desiccated seed (Job et al., 2005). Following imbibition, at least some LEA proteins, when hydrated, would lose any structural features helping to induce a fit with their client proteins, leaving their client proteins more susceptible to oxidation, which was the case for proteins involved in translation upon seed hydration (Job et al., 2005).

Using a Tandem Affinity Purification approach repeated four times (twice in the light and twice in the dark) Shaw (2016) recovered and identified a group of client proteins binding to a TAP-LEA5 protein (At4g02480, which is LEA38 of the LEA3 family; Hundertmark and Hincha, 2008). Of the five client proteins recovered two or more times using this approach, two were involved in translation and these were recovered most consistently from the screen. The Arabidopsis DEAD-Box RNA Helicase 22 (RH22) (Category 3) was recovered each time the assay was performed. RH22 is required for proper maturation and assembly of plastidial ribosomal RNA crucial for translation in the organelle (Chi et al., 2012). The next most consistently recovered client protein (three out of four times) was PUMILIO24, an RNA binding protein of the PUF family (Tam et al., 2010), also involved in ribosomal RNA maturation (Category 3) and assembly in the nucleolus (Shanmugam et al., 2017; Maekawa et al., 2018). Although these efforts to identify legitimate, conspecific LEA protein client proteins have indicated, for two different families of LEAs, that proteins involved in translation are preferred targets, many LEA proteins are known to bind membranes rather than proteins (Thalhammer et al., 2010; Tolletter et al., 2010; Eriksson et al., 2011). Additionally, for those LEA proteins that do bind proteins, it is still unclear
whether proteins involved in translation are an ubiquitous set of client proteins or if the two LEA proteins investigated thus far coincidentally had proteins involved in translation as their targets. Regardless, what is clear is that some proteins of the translational apparatus are preferred client proteins bound (potentially protected) by some members of the LEA family, entirely consistent with Job’s rule.

Protection: Difficulty assigning protection of protein associated with translation to altered soluble carbohydrate profiles

The alteration of the sugar profile during dehydration of tissues is a well-documented occurrence in pollen, seeds and resurrection plants (Griffiths et al., 2014). One of the attributes of increasing sugar concentration concomitant with water loss is the transition to an amorphous state in the cytoplasm, and presumably, the organelles in which the cellular constituents are embedded. The increase in viscosity is sufficient to greatly impede diffusion rates while dampening metabolism to such an extent that it is difficult to measure. Carbohydrate-mediated protection is thought to be a general phenomenon (ElSayed et al., 2014) encompassing both membranes and proteins that, although not preferentially focused on proteins (let alone proteins involved in translation), by protecting proteins promiscuously, protects those of the translational apparatus as well (Buera et al., 2004).

There are some cryptic correlations between the abundance of sugars in stressed cells and desiccation tolerance. One of the relationships that has been gaining attention is the importance of the ratio between the raffinose family oligosaccharides (RFO) and sucrose (first identified by Chen and Burris, 1990) in the acquisition of desiccation tolerance (Pereira Lima et al., 2017) and/or enhancement of seed longevity (Li et al., 2017b). In vitro studies suggest that the mixture of the sugars may not benefit protein longevity or desiccation tolerance, potentially diverting research efforts to examine how the sugar mixture may influence membrane stabilization and/or the propensity of the mixture to remain amorphous (Davidson and Sun, 2001). However, it is still possible that a favourable RFO/sucrose ratio may protect proteins in vivo due to the complexity of sugar metabolism in the cell being beyond the capacity of in vitro studies to unveil. For instance, trehalose abundance in stressed yeast cells has been linked to the propensity of these cells to reduce protein aggregation generally. Up-regulation of trehalose production has been demonstrated to result in increased heat shock protein (HSP104; a Category 3 chaperonin) abundance, and increased autophagic clearing of protein aggregates (Chaudhary et al., 2014). The beneficial influence in preventing protein aggregation was demonstrated to be, in part, the dual action of trehalose and HSP104 when they were present concurrently and in a strict stoichiometry (Saleh et al., 2014) that was not entirely dependent on the up-regulation of autophagy by trehalose accumulation. This level of synergy would not be evident with in vitro studies. However, none of these features of RFO/sucrose ratios or trehalose-mediated protection (chaperonin-induction, synergistic action, or autophagy stimulation) has been categorically identified as specifically targeted to proteins of the translational apparatus.

Repair: Protein repair pathways

Certain AAs in a polypeptide are prone to damage through oxidation, isomerization and spontaneous conversion. For some of these forms of damage, cellular repair mechanisms have been identified in most forms of life, testifying to both the ubiquity of the agents of damage (e.g. oxidation; Gracy et al., 1999) as well as to the importance of rectifying damage, when possible, to maintain a functional proteome (Clarke, 2003). Following the discovery, using radio-labelling and two-dimensional gel electrophoresis (2DGE), that seed proteins involved in translation decreased in abundance 1 day after imbibition in the proteome of aged – relative to unaged – seeds (Rajjou et al., 2008), it was noted (again using 2DGE, membrane transfer, and on-blot methylation) that there were certain proteins acting as preferred substrates of the protein repair enzyme PROTEIN ISOASPARTYL METHYLTRANSFERASE (PIMT) (Dinkins et al., 2008).

Repair: PROTEIN ISOASPARTYL METHYLTRANSFERASE (PIMT)

Isoaspartate (IsoAsp) formation is a common form of damage incurred under physiological conditions (Geiger and Clarke, 1987) and its non-enzymatic production (or at least the production of its immediate succinimidyl precursor) is heightened under stressful conditions (Mudgett and Clarke, 1994) and in desiccated, aged tissues (Mudgett et al., 1997). There are numerous studies demonstrating increased seed longevity due to up-regulation of PIMT or a reduction in this seed attribute upon pimt dysfunction (Oge et al., 2008; Verma et al., 2013; Wei et al., 2015). In an endeavour to identify those proteins in seeds that were particularly prone to isoAsp formation and/or preferentially repaired by the PIMT enzyme, many proteins involved in translation were identified as preferred PIMT targets (or particularly susceptible to isoAsp formation; Chen et al., 2010). These included proteins responsible for ribosomal maturation (AT3G51270; AT5G62190; Category 3); a component of the mitochondrial inner membrane translocon (AT5G51150; Category 8); rRNA modification (AT5G55920; Category 2); and an r-protein (AT3G22230; Category 1). Moreover, this was in stark contrast with PIMT target proteins identified in organisms incapable of surviving desiccation (Reissner et al., 2006; Zhu et al., 2006; Dai et al., 2013). In these desiccation-sensitive organisms, with a large number of PIMT target proteins identified, there is only a single report of a protein (eIF4E-binding protein2; Category 7) intimately associated with the eukaryotic translational complex, being a target of PIMT (Bidinosti et al., 2010).

Of the enzymes involved in translation that were preferentially repaired by PIMT in screens of the seed proteome, one of those involved in the processing of the ribosomal RNAs in the nucleolus (Category 3) was examined further (Galland and Rajjou, 2015; Huang et al., 2016a; Lorkovic et al., 1997; Nayak et al., 2013). An orthologue of this Arabidopsis DEAD-box RNA helicase (PLANT RNA HELICASE75, PRH75) had been shown to be the product of one of the few transcripts preferentially up-regulated in imbibed aged seeds of mung bean (Vigna radiata) relative to unaged seeds (Li et al., 2001). This up-regulation of transcript abundance may be a reaction to a decline in active protein abundances, similar to the findings in imbibed, aged Arabidopsis seeds (Rajjou et al., 2008) where a DEAD-box RNA helicase, involved in mRNA export (Category 4; Kammel et al., 2013) declines in aged seeds. Further studies in Arabidopsis determined that most prh75 mutants are embryo lethal and that weakly penetrant prh75 mutations, while viable, produced abnormally shaped (corkscrewed) seeds/embryos (or those with compromised completion of germination) at high
frequency (Nayak et al., 2013; Huang et al., 2016b), underlining how crucial the functionality of PRH75 is during plant development, a functionality safeguarded by PIMT (Nayak et al., 2013). Indeed, plants possess two different genes encoding PIMT (Xu et al., 2004) and dysfunction of one is sufficient to severely compromise seed longevity (Oge et al., 2008; Verma et al., 2013).

**Repair: METHIONINE SULFOXIDE REDUCTASE**

If the proteins of the translational apparatus are preferentially repaired (at least by PIMT and Met Sulfoxide Reductase; Caldwell et al., 1978; Chen et al., 2010; Nayak et al., 2013), this may be a contributing reason why they are among the more resilient proteins in the cytoplasm (Nelson et al., 2014). At least isoAsp is relegated as problematic to its occurrence in a peptide chain. Oxidation of Met, on the other hand, can occur in a protein as well as to free methionine, or that bound as an aminoacyl-tRNA (Chousterman and Chapelle, 1981). In fact, oxidative stress has been shown to result in the preferential mis-acetylation of methionine to non-methionine tRNAs (Category 6) that, coupled with an oxidation-mediated reduction in the efficiency of AMINOACYL-TRNA SYNTHETASE editing capacity, decreases the fidelity of translation (Ling and Soll, 2010). Such ‘controlled inaccuracy’ (Lee et al., 2014) is mediated through an oxidative environment leading to phosphorylation of key residues in the METHIONYL-trNA SYNTHETASE (Category 5) that render it less discriminating in the tRNAs perceived as cognate (Lee et al., 2014). Due to the hypothesized role of solvent-exposed methionines acting as a ROS reductant (Bender et al., 2008), it has been postulated that this mis-priming of tRNAs to increase MET incidence in the proteome, is a cryptic means by which cells protect themselves from oxidative stress (Netzer et al., 2009). Moreover, free, oxidized methionine can form diastereomers (Met-S-SO and Met-R-SO) for which metazoans and higher plants have not retained the enzyme capable of efficiently reducing Met-S-SO (Le et al., 2009). Met-SO will not form S-adenosyl methionine (Achilli et al., 2015), the primary methyl donor in the cell, and the means through which PIMT functions to convert isoAsp to Asp.

While these damages impact translation generally, there is at least one occurrence of an oxidized methionine in an r-protein (Category 1) that is known to inhibit translation specifically but that can be repaired to regain functionality (Caldwell et al., 1978). The reduction of both free methionine-sulfoxide and methionine-sulfoxide present in a polypeptide context is, obviously, an important facet of Job’s rule. While METHIONYL-trNA SYNTHETASE cannot be charged with MET-SO (Category 6) (Lemoine et al., 1968), Met can be oxidized once it has formed the aminoacyl trNA^{MET-SO} (Category 5; Chousterman and Chapelle, 1981), so reduction to tRNA^{MET} is also an important aspect of Job’s rule.

**Repair: Chaperonins: PEPTIDYL-PROLYL CIS-TRANS ISOMERASE**

In addition to being oxidized, proline can isomerize to/from trans/cis-isomers, important for protein folding (Wedemeyer et al., 2002). Peptidyl-prolyl cis-trans isomerases (PPIases) are a class of enzyme capable of isomerizing proline between cis- and trans-isomers at the N-terminal amide bond in proteins (Wedemeyer et al., 2002; Camilloni et al., 2014). Proline isomerization has been documented to influence seed vigour through mutation of specific PPIases (Category 7; Bissoli et al., 2012). The ribosome inserts all prolines in the trans conformation (Feige et al., 2010), requiring PPIases to subsequently convert the trans proline to cis, if necessary, to fold the nascent polypeptide. Nevertheless, in yeast, concurrent elimination of 12 cyclophillin and FK506 binding proteins (PPIase enzymes) through mutagenesis was not lethal (Dolinski et al., 1997), leaving the authors to propose that each PPIase has a unique set of proteins on which it acts in yeast. Later studies recovered specific protein interactors with FK506 binding protein 12 (FKBP12) prolyl isomerase, a cyclophillin known to bind FK506 and cyclosporine, validating the preposition of the authors that PPIases will have specific client proteins with which they react (Dolinski and Heitman, 1999). However, to date, no protein associated with translation has been identified in any organism as specifically interacting with a PPIase in order to be refolded following stress.

In bacteria, the TRIGGER FACTOR chaperonin protein with PPIase activity, is physically associated with the 50S ribosomal-tunnel from which the amino-terminus of the protein being constructed emerges and is the first chaperonin the nascent polypeptide encounters (Kristensen and Gajhede, 2003). In plants, the plastid is apparently the only compartment supporting translation that has retained a TRIGGER FACTOR chaperonin (Category 7; Ries et al., 2017) which, due to its susceptibility to supraoptimal temperature stress, is thought to confer to plastidial translation some advantage during heat stress (Ries et al., 2017). While none of the above mentioned isomerizations has been considered ‘damage’, or to occur preferentially in proteins involved in translation, in plants it does link PPIase activity tightly to translation, at least in the plastid.

**Protection/repair: Chaperonins: heat shock proteins**

In developing soybean seeds, transcripts encoding certain HEAT SHOCK PROTEINS (HSPs) accumulate late during embryogenesis and are remarkable as among the most positively correlated with seed longevity (Pereira Lima et al., 2017). As with GALACTINOL SYNTHASE over-expression studies, there are numerous examples where over-expression of HSPs or the genes encoding transcription factors (HEAT SHOCK FACTORS), up-regulating the plant HSP arsenal, results in increased seed longevity after artificial ageing (Priedo-Dapena et al., 2006; Personat et al., 2014; Kaur et al., 2015). The opposite has also been demonstrated where mutated HSF genes, introduced from sunflower into tobacco, have led to the down-regulation of HSPs and a concomitant reduction in seed tolerance of accelerated ageing conditions (Tejedor-Cano et al., 2010). Similarly, reduction of HSP101 though mutagenesis, while producing viable seeds and apparently normal plants in the absence of stress, displayed a reduction in seed resistance to thermal insult during germination (Hong and Vierling, 2000). While their roles protecting nascent proteins from misfolding and capacity to catalyse proper protein configurations upon folding make them undeniably associated with proteins associated with translation (and justify their title above as protective Chaperonin proteins), are there instances where such chaperonins are known to preferentially assist the refolding of proteins of the translational apparatus following damage (justifying their title as repair Chaperonin proteins)? The HSP90 has been shown to have as substrates other HSPs, as well as cyclophilins (Category 3), translational elongation factor kinases (Category 7), and aminoacyl-t-RNA synthetase complexes (Category 6) (Picard, 2002). The bacterium Synecochystis, when subjected to heat stress, up-regulated HSP16.6 that bound an r-protein.
Such repair is anticipated to be extended to periods of stress during which subunits were preferentially repaired to become resoluble. Of these proteins rendered insoluble upon heat shock, phase, following recovery from heat shock (McLoughlin et al., 2016), Proteins that associated with both HSP17.4 and HSP17.6 were identified as the subunits of eEF1B (a, b and g, Category 7) (McLoughlin et al., 2016). Furthermore, these authors stringently examined the capacity of the eEF1B subunits to be: (1) denatured during heat shock with a proportion becoming insoluble; and (2) renatured, re-entering the soluble phase, following recovery from heat shock (McLoughlin et al., 2016). Of these proteins rendered insoluble upon heat shock, eEF1B subunits were preferentially repaired to become resoluble. Such repair is anticipated to be extended to periods of stress during germination (Fig. 1) and potentially to recovery following imbibition, although these investigations remain to be performed. Their conclusion was that these proteins, essential for translation, are preferred client proteins of the two small HSPs (McLoughlin et al., 2016), a view entirely consistent with Job’s rule.

Conclusion

We have investigated evidence supporting Job’s rule (the proteins involved in translation must endure desiccation, quiescence, and rehydration in a functional state if the seed is to survive), highlighting data indicating that proteins of the translational apparatus are particularly resilient. Studies comparing the identities of proteins oxidized in dry – relative to imbibed – seeds suggest that proteins associated with translation are somewhat protected from oxidation in the dry state, although little is known about how oxidation of proteins of the translational apparatus is mitigated while desiccated, or how they exhibit enhanced resiliency. There is, however, some evidence of innate protein supercharging of r-proteins, involved in translation, at biologically relevant pH that may be relevant to these observations. Furthermore, endeavours to identify preferred client proteins bound by LEA proteins or repaired by PIMT or chaperonin networks, has provided evidence supportive of Job’s rule because these systems are seemingly skewed towards serving the proteins of the translational apparatus. The more information obtained on the identities of client proteins preferentially serviced by conspecific protection and repair mechanisms the greater the opportunity to identify: (1) what proteins are susceptible to damage; and (2) what parts of these proteins are particularly at risk of damage. This knowledge is edifying to efforts to understand fundamental constraints to anhydrobiosis and practically, could lead to re-engineering proteins to make them less susceptible to a plethora of damage.

Acknowledgements. The authors wish to thank the USDA Western Section Multistate Research Project Working Group W-3168 ‘Environmental and Genetic Determinants of Seed Quality and Performance’, the International Society for Seed Science (ISSS), and Western Section American Society of Plant Biologists for providing the opportunity to present aspects covered in this manuscript at the ISSS triennial meeting, Monterey, California, USA. Particular thanks are due to Henk Hilhorst, Editor of the ISSS journal, Seed Science Research, for providing space for articles such as this to highlight the ISSS triennial meeting topics. Hatch funds were used to support the project detailed in this communication for which we are grateful.

References

Achilli C, Ciana A and Minetti G (2015) The discovery of methionine sulfone reductase enzymes: an historical account and future perspectives. Biofactors 41, 135–152.

Akter S, Huang J, Waszczak C, Jacques S, Gevaert K, Van Breusegem F and Messens J (2013) Cysteines under ROS attack in plants: a proteomics view. Journal of Experimental Botany 66, 2935–2944.

Alpert P (2005) The limits and frontiers of desiccation-tolerant life. Integrative and Comparative Biology 45, 685–695.

Apel K and Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology 55, 373–399.

Bai B, Novak O, Jliang K, Hanson J and Bentsink L (2018) Combined transcriptome and translatome analyses reveal a role for tryptophan-dependent auxin biosynthesis in the control of DOG1-dependent seed dormancy. New Phytologist 217, 1077–1085.

Bai B, Peviani A, van der Horst S, Gamm M, Snel B, Bentsink L and Hanson J (2017) Extensive translational regulation during seed germination revealed by polysomal profiling. New Phytologist 214, 233–244.

Bailly C (2004) Active oxygen species and antioxidants in seed biology. Seed Science Research 14, 93–107.

Bailly C, El-Marouf-Bouteau H and Corbeille F (2008) From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. Comptes Rendus Biologies 331, 806–814.

Barakat A, Szick-Miranda K, Chang IF, Guyot R, Blanc G, Cooke R, Delseny M and Bailey-Serres J (2001) The organization of cytoplasmic ribosomal protein genes in the Arabidopsis genome. Plant Physiology 127, 398–415.

Babouss-Serhal I, Soubigou-Taconnat L, Bailly C and Leymarie J (2015) Germination potential of dormant and nondormant arabidopsis seeds is driven by distinct recruitment of messenger rnas to polysomes. Plant Physiology 168, 1049–1065.

Basha E, Lee GJ, Breci LA, Hauarath AC, Buan NR, Giese KC and Vierling E (2004) The identity of proteins associated with a small heat shock protein during heat stress in vivo indicates that these chaperones protect a wide range of cellular functions. Journal of Biological Chemistry 279, 7566–7575.

Battaglia M, Olivera-Carrillo Y, GarciaRubio A, Campos F and Covarrubias AA (2008) The enigmatic LEA proteins and other hydrophilins. Plant Physiology 148, 6–24.

Beltran-Pena E, Ortiz-Lopez A and Sanchez de Jimenez E (1995) Synthesis of ribosomal proteins from stored mRNAs early in seed germination. Plant Molecular Biology 28, 327–336.

Bender A, Hajieva P and Moosmann B (2008) Adaptive antioxidant methionine accumulation in respiratory chain complexes explains the use of a deviant genetic code in mitochondria. Proceedings of the National Academy of Sciences of the USA 105, 16496–16501.

Berjak P (2006) Unifying perspectives of some mechanisms basic to desiccation tolerance across life forms. Seed Science Research 16, 1–15.

Bidinosti M, Martineau Y, Frank F and Sonenberg N (2012) Repair of isopentenyl-prolyl cis-trans isomerase ROF2 modulates intracellular pH homeostasis in Arabidopsis. Plant Journal 70, 704–716.

Boisvert FM, Ahmad Y, Gierlinski M, Charriere F, Lamont D, Scott M, Barton G and Lamond AI (2012) A quantitative spatial proteomics analysis of proteome turnover in human cells. Molecular and Cellular Proteomics 11, M111 01429.

Boucher V, Buittak J, Lin X, Boudet J, Hoekstra FA, HummertMark M, Renard D and Leprince O (2010) MtPM25 is an atypical hydrophobic
late embryogenesis-abundant protein that dissociates cold and desiccation-aggregated proteins. Plant Cell and Environment 33, 418–430.

Browne J, Tunncliffe A and Burnell A (2002) Anhydrobiosis: plant desiccation gene found in a nematode. Nature 416, 38.

Browne JA, Dolan KM, Tyson T, Goyal K, Tunncliffe A and Burnell AM (2004) Dehydration-specific induction of hydrophilic protein genes in the anhydrobiotic nematode *Aphelenchus avenae*. *Eukaryotic Cell* 3, 966–975.

Buera P, Schebor C and Elizalde B (2004) Effects of carbohydrate crystalization on stability of dehydrated foods and ingredient formulations. *Journal of Food Engineering* 67, 157–165.

Buitink J, Claessens MM, Hemminga MA and Hoekstra FA (2004) Influence of water content and temperature on molecular mobility and intracellular glasses in seeds and pollen. *Plant Physiology* 118, 531–541.

Buitink J, Hemminga MA and Hoekstra FA (2000) Is there a role for oligosaccharides in seed longevity? An assessment of intracellular glass stability. *Plant Physiology* 122, 1217–1224.

Buitink J and Leprioce O (2004) Glass formation in plant anhydrobiotes: survival in the dry state. *Cryobiology* 48, 215–228.

Calder R, Luk GC, Weissbach H and Brot N (1978) Oxidation of the methionine residues of Escherichia coli ribosomal protein L12 decreases the protein’s biological activity. *Proceedings of the National Academy of Sciences of the USA* 75, 5349–5352.

Camilloni C, Sahakyan AB, Holliday MJ, Isern NG, Zhang F, Eisemmseder EZ and Vendruscolo M (2014) Cyclophilin A catalyzes protein line isomerization by an electrostatic handle mechanism. *Proceedings of the National Academy of Sciences of the USA* 111, 10203–10208.

Candat A, Paszkiewicz G, Neveu M, Gautier R, Logan DC, Avelange-Macherel MH and Macherel D (2014) The ubiquitous distribution of late embryogenesis abundant proteins across cell compartments in Arabidopsis offers tailored protection against abiotic stress. *Plant Cell* 26, 3148–3166.

Carroll AJ (2013) The arabidopsis cytosolic ribosomal proteins: from form to function. *Frontiers in Plant Science* 4, 32.

Carroll AJ, Heazlewood JL, Ito J and Millar AH (2008) Analysis of the Arabidopsis cytosolic ribosome proteome provides detailed insights into its components and their post-translational modification. *Molecular and Cellular Proteomics* 7, 347–369.

Chakrabortee S, Tripathi R, Watson M, Schierle GS, Kurniawan DP, Kaminski CF, Wise MJ and Tunncliffe A (2012) Intrinsically disordered proteins as molecular shields. *Molecular BioSystems* 8, 210–219.

Chaudhary RK, Kardani J, Singh K, Banerjee R and Roy I (2014) Deciphering the roles of trehalose and Hsp104 in the inhibition of aggregation of mutant huntingtin in a yeast model of Huntington’s disease. *Neuromolecular Medicine* 16, 280–291.

Cheah KS and Osborne DJ (1978) DNA lesions occur with loss of viability in embryos of ageing eye seed. *Nature* 272, 593–599.

Chen T, Nayak N, Majee SM, Lowenson J, Schafermeyer KR, Eliopoulos AG, Lloyd TD, Dinkins R, Perry SE, Forsthoefer NR, Clarke SG, Vernon DM, Zhou ZS, Rejtar T and Downie AB (2013) Evidence for the absence of enzymatic reactions in the glassy state. *Proceedings of the National Academy of Sciences of the USA* 110, 15752–15757.

Chen Y and Burris JS (1990) Role of carboxylates in desiccation tolerance and membrane behavior in maturing maize seed. *Crop Science* 30, 971–975.

Chi W, He B, Mao J, Li Q, Ma J, Ji D, Zou M and Zhang L (2012) The function of RH22, a DEAD RNA helicase, in the biogenesis of the 50S ribosomal subunit. *Molecular Biology* 158, 693–707.

Chousterman S and Chapelle F (1981) Chemical modifications of amino acids exterfied to tRNA (other than acylations), pp. 103–106 in Moldave K (ed), *RNA and Protein Synthesis*. New York: Academic Press.

Christian R, Nagaraj N, Frohlich F and Walther TC (2014) Global proteome turnover analyses of the yeasts *S. cerevisiae* and *S. pombe*. *Cell Reports* 9, 1959–1965.

Clarke S (2003) Ageing as war between chemical and biochemical processes: protein methylation and the recognition of age-damaged proteins for repair. *Ageing Research Reviews* 2, 263–285.

Clegg JS (2001) Cryptobiosis – a peculiar state of biological organization. *Comparative Biochemistry and Physiology: B Biochemical and Molecular Biology* 128, 613–624.

Clerkx EJ, El-Lithy M, Vierling E, Ruys GJ, Blanketijn-De Vries H, Groot SP, Vreugdenhil D and Koornneef M (2004) Analysis of natural allelic variation of Arabidopsis seed germination and seed longevity traits between the accessions Landsberg erecta and Shakdara, using a new recombinant inbred line population. *Plant Physiology* 135, 432–443.

Costa MD, Cooper K, Hillhorst HWM and Farrant JM (2017) Orthodox seeds and resurrection plants: two of a kind? *Plant Physiology* 175, 589–599.

Crowe JH, Carpenter JF and Crowe LM (1998) The role of vitrification in anhydrobiosis. *Annual Review of Physiology* 60, 73–103.

Dai S, Ni W, Panatanan AN, Clarke SG, Karger BL and Zhou ZS (2013) Integrated proteomic analysis of major isoaspartyl-containing proteins in the urine of wild type and protein L-isoaspartate O-methyltransferase-deficient mice. *Analytical Chemistry* 85, 2423–2430.

Davidson P and Sun WQ (2001) Effect of sucrose/raffinose mass ratios on the stability of co-lyophilized protein during storage above the Tg. *Pharmacological Research* 18, 474–479.

Debeaujon I, Leon-Kloosterziel KM and Koornneef M (2000) Influence of the testa on seed dormancy, germination, and longevity in Arabidopsis. *Plant Physiology* 122, 403–414.

Dendoy E, Schyns R, Deltour I and Verly WG (1987) Appearance and repair of apurinic/apyrimidinic sites in DNA during early germination. *Mutation Research* 181, 57–60.

Dinkins RD, Majee SM, Nayak NR, Martin D, Xu Q, Belcastro MP, Houtzi BL, Beach CM and Downie AB (2008) Changing transcriptional initiation sites and alternative 5′- and 3′-splice site selection of the first intron deports Arabidopsis protein isoaspartyl methyltransferase2 variants to different subcellular compartments. *Plant Journal* 55, 1–13.

Dolinski K, Muir S, Cardenas M and Heitman J (1997) All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in Saccharomyces cerevisiae. *Proceedings of the National Academy of Sciences of the USA* 94, 13093–13098.

Dolinski KJ and Heitman J (1999) Hm10p, a high mobility group 1/2 homolog, is genetically and physically interacts with the yeast FKBP12 prolyl isomerase. *Genetics* 151, 935–944.

Doria E, Galleschi L, Calucchi L, Pinzino C, Piu R, Cassani E and Nielsen E (2009) Phytic acid prevents oxidative stress in seeds: evidence from a maize (*Zea mays L.*) low phytic acid mutant. *Journal of Experimental Botany* 60, 967–978.

El-Maarouf-Bouteu H, Meimoun P, Job C, Job D and Bailly C (2013) Role of protein and mRNA oxidation in seed dormancy and germination. *Frontiers in Plant Science* 4, 77.

Ellis RH, Hong TD and Roberts EH (1988) A low-moisture-content limit to logarithmic relations between seed moisture content and longevity. *Annals of Botany* 61, 405–408.

ElSayed AI, Rafudeen MS and Golldack D (2014) Physiological aspects of raffinose family oligosaccharides in plants: protection against abiotic stress. *Plant Biology* 16, 1–8.

Eriksson S, Eremina N, Barth A, Danielsson J and Harryson P (2016) Membrane-induced folding of the plant stress dehydrin Lti30. *Plant Physiology* 171, 932–943.

Eriksson SK, Kutzer M, Procek J, Grobben G and Harryson P (2011) Tunable membrane binding of the intrinsically disordered dehydrin Li30, a cold-induced plant stress protein. *Plant Cell* 23, 2391–2404.

Fedyukina DV, Jennaro TS and Cavagnero S (2013) Charge segregation and low hydrophobicity are key features of ribosomal proteins from different organisms. *Journal of Biological Chemistry* 289, 6740–6750.

Feige MJ, Hendershot LM and Buchner J (2010) How antibodies fold. *Trends in Biochemical Sciences* 35, 189–198.

Fernandez-Marin B, Kraner I, San Sebastian M, Artetxe U, Laza JM, Vilas JL, Pritchard HW, Nadajaran J, Miguez F, Becerril JM and Garcia-Plazaola JI (2013) Evidence for the absence of enzymatic reactions in the glassy state. A case study of xanthophyll cycle pigments in the desiccation-tolerant moss *Symnichium ruralis*. *Journal of Experimental Botany* 64, 3033–3043.

Fleming MB, Richards CM and Walters C (2017) Decline in RNA integrity of dry-stored soybean seeds correlates with loss of germination potential. *Journal of Experimental Botany* 68, 2219–2230.

Gachomo EW, Jimenez-Lopez JC, Baptiste LJ and Kotchoni SO (2014) GIGANTUS1 (GTS1), a member of Transducin/WD40 protein superfamily, 177
controls seed germination, growth and biomass accumulation through ribosome-biogenesis protein interactions in Arabidopsis thaliana. BMC Plant Biology 14, 37.

Galland M, Huguet R, Arc E, Cuffe G, Job D and Rajou I (2014) Dynamic proteomics emphasizes the importance of selective mRNA translation and protein turnover during Arabidopsis seed germination. Molecular and Celluar Proteomics 13, 252–268.

Galland M and Rajou I (2015) Regulation of mRNA translation controls seed germination and is critical for seedling growth. Frontiers in Plant Science 6, 284.

Gao F, Rampitsch C, Chitnis VR, Humphreys GD, Jordan MC and Ayle BT (2013) Integrated analysis of seed proteome and mRNA oxidation reveals distinct post-transcriptional features regulating dormancy in wheat (Triticum aestivum L.). Plant Biotechnology Journal 11, 921–932.

Geiger T and Clarke S (1987) Deamidation, isomerization, and racemization at asparaginyl and aspartyl residues in peptides. Succinimide-linked reactions that contribute to protein degradation. Journal of Biological Chemistry 262, 785–794.

Gong X, Jiang Q, Xu J, Zhang J, Teng S, Lin D and Dong Y (2013) Disruption of the rice plastid ribosomal protein s20 leads to chloroplast developmental defects and seedling lethality. G3 3, 1769–1777.

Goya K, Walton IJ and Tunnalcliffe A (2005) LEA proteins prevent protein aggregation due to water stress. Biochem Journal 388, 151–157.

Goy RW, Talent JM, Kong Y and Conrad CC (1999) Reactive oxygen species: the unavoidable environmental insult? Mutation Research 428, 17–22.

Griffiths CA, Gaff DF and Neale AD (2014) Drying without senescence in resurrection plants. Frontiers in Plant Science 5, 36.

Grimaud R, Ezraty B, Mitchell JK, Lafitte D, Briand C, Derrick PJ and Barras F (2001) Repair of oxidized proteins. Identification of a new nitrosamine sulfoxide reductase. Journal of Biological Chemistry 276, 48915–48920.

Grosea H, Breton M, Sirand-Pugnet P, Tardy F, Thiaucourt F, Citti C, Barre A, Yoshizawa S, Fourmy D, de Crecy-Lagard V and Blanchard A (2014) Predicting the minimal translation apparatus: lessons from the reductive evolution of mloclotes. PLoS Genetics 10, e1004363.

Hara M, Fujinaga M and Kuboi T (2004) Radical scavenging activity and oxidative modification of citrus dehydrin. Plant Physiology and Biochemistry 42, 657–662.

Haslebeck M and Vierling E (2015) A first line of stress defense: small heat shock proteins and their function in protein homeostasis. Journal of Molecular Biology 427, 1537–1548.

Hengherr S, Heyer AG, Kohler HR and Schill RO (2008) Trehalose and anhydrobiosis in tardigrades – evidence for divergence in responses to dehydration. FEBS Journal 275, 281–288.

Hernandez-Sanchez IE, Maruri-Lopez I, Graether SP and Jimenez-Bremont JF (2017) In vivo evidence for homo- and heterodimeric interactions of Arabidopsis thaliana dehydrins A1COR47, A1ERD10 and A1RAR1B. Scientific Reports 7, 17036.

Hodge JE (1953) DEHYDRATED FOODS. Chemistry of Browning reactions in model systems. Agricultural and Food Chemistry 1, 928–943.

Holmgren A, Johansson C, Berndt C, Lonn ME, Hudemann C and Liljestrand C (2005) Thiol redox control via thioredox and glutaredoxin systems. Biochemical Society Transactions 33, 1375–1377.

Hong SW and Vierling E (2000) Mutants of Arabidopsis thaliana defective in the acquisition of tolerance to high temperature stress. Proceedings of the National Academy of Sciences of the USA 97, 4392–4397.

Huang CK, Shen YL, Huang LF, Wu SJ, Yeh CH and Lu CA (2016a) The DEAD-Box RNA Helicase AtRAB18 participates in pre-rRNA processing, plant development and cold tolerance in Arabidopsis. Plant and Cell Physiology 57, 174–191.

Huang CK, Sie YS, Chen YF, Huang TS and Lu CA (2016b) Two highly similar DEAD box proteins, OsRHS2 and OsRHS34, homologous to eukaryotic initiation factor 4AIII, play roles of the exon junction complex in regulating growth and development in rice. BMC Plant Biology 16, 84.

Huang Z, Boubriak I, Osborne DJ, Dong M and Gutterman Y (2008) Possible role of pectin-containing mucilage and dew in repairing embryonic DNA of seeds adapted to desert conditions. Annals of Botany 101, 277–283.

Hummel M, Dobrenel T, Cordewener JJ, Davanture M, Meyer C, Smeeckens SJ, Bailey-Serres J, America TA and Hanson J (2015) Proteomic LC-MS analysis of Arabidopsis cytosolic ribosomes: identification of ribosomal protein paralogs and re-annotation of the ribosomal protein genes. Journal of Proteomics 128, 436–449.

Hundertmark M, Buitink J, Leprince O and Hincha DK (2011) The reduction of seed-specific dehydrins reduces seed longevity in Arabidopsis thaliana. Seed Science Research 21, 165–173.

Hundertmark M and Hincha DK (2008) LEA (late embryogenesis abundant) proteins and their encoding genes in Arabidopsis thaliana. BMC Genomics 9, 118.

Isbell HS and Frush HJ (1958) Mutarotation, hydrolysis, and rearrangement reactions of glycrolamines. Journal of Organic Chemistry 23, 1309–1319.

Job C, Rajou I, Lovigny Y, Belghazi M and Job D (2005) Patterns of protein oxidation in Arabidopsis seeds and during germination. Plant Physiology 138, 790–802.

Kamek C, Thomaier M, Sorensen BB, Schubert T, Langst G, Grasser M and Grasser KD (2013) Arabidopsis DEAD-box RNA helicase UAP56 interacts with both RNA and DNA as well as with mRNA export factors. PLoS One 8, e60644.

Kaur H, Petla BP, Kamble NU, Singh A, Rao V, Salvi P, Ghosh S and Majeed M (2015) Differentially expressed seed aging responsive heat shock protein OsHSP18.2 implicates in seed vigor, longevity and improves germination and seedling establishment under abiotic stress. Frontiers in Plant Science 6, 713.

Kester ST, Greene RL and Houtz RL (1997) Priming and accelerated aging affect L-isoaspartyl methyltransferase activity in tomato (Lycopersicon esculentum Mill.). seed. Journal of Experimental Botany 309, 943–949.

Kim I, Hempel FD, Sha K, Pfuger J and Zambryski PC (2002) Identification of a developmental transition in plasmodesmatal function during embryogenesis in Arabidopsis thaliana. Development 129, 1261–1272.

Kovacs D, Kalmar E, Torok Z and Tompa P (2008) Chaperone activity of ERD10 and ERD14, two disordered stress-related plant proteins. Plant Physiology 147, 381–390.

Kraner I, Cram WJ, Zorn M, Wornik S, Yoshimura I, Stabentheiner E and Pfeiffer HW (2005) Antioxidants and photoprotection in a lichen as compared with its isolated symbiotic partners. Proceedings of the National Academy of Sciences of the USA 102, 3141–3146.

Kravchenko A, Citierne S, Jehanno I, Bersimbaev RI, Veit B, Meyer C and Leprince AS (2015) Mutations in the Arabidopsis LST8 and Raptor genes encoding partners of the TOR complex, or inhibition of TOR activity decrease asicid acid (ABA) synthesis. Biochemical and Biophysical Research Communications 467, 992–997.

Kristensen O and Gajhede M (2003) Chaperone binding at the ribosomal exit tunnel. Structure 11, 1547–1556.

Kushwaha R, Downie AB and Payne CM (2013) Uses of phyte dyse in agriculture: sequence analysis and comparative modeling of late embryogenesis abundant client proteins suggest protein-nuclear acid binding functionality. Computational and Mathematical Methods in Medicine 470390. http://dx.doi.org/10.1155/2013/653759.

Kushwaha R, Lloyd TD, Schafermeyer KR, Kumar S and Downie AB (2012) Identification of Late Embryogenesis Abundant (LEA) protein putative interactors using phyte dyse. International Journal of Molecular Science 13, 6582–6603.

Lawrence MS, Phillips KJ and Liu DR (2007) Supercharging proteins can impart unusual resilience. Journal of the American Chemical Society 129, 10110–10112.

Le DT, Lee BC, Marino SM, Zhang Y, Fomenko DE, Kaya A, Haciguleli E, Kwak GH, Koc A, Kim HY and Gladyshev VN (2009) Functional analysis of free methionine–R-sulfoxide reductase from Saccharomyces cerevisiae. Journal of Biological Chemistry 284, 4354–4364.

Lee YJ, Kim DG, Kim BG, Hong J, Kang T, Oh YS, Kim KR, Han BW, Hwang BJ, Kang BS, Kang MS, Kim MH, Kwon NH and Kim S (2014) Promiscuous methionyl-tRNA synthetase mediates adaptive mistranslation to protect cells against oxidative stress. Journal of Cell Science 127, 4234–4245.

Lemoine F, Waller JP and van Rapenbusch R (1968) Studies on methionyl transfer RNA synthetase. 1. Purification and some properties of methionyl transfer RNA synthetase from Escherichia coli K-12. European Journal of Biochemistry 4, 213–221.
Leroy E, Harren FJ, Buitink J, Alberda M and Hoekstra FA (2000) Metabolic dysfunction and unabated respiration precede the loss of membrane integrity during dehydration of germinating radicles. *Plant Physiology* 122, 597–608.

Levine RL, Moskovitz J and Stadtman ER (2000) Oxidation of methionine in proteins: roles in antioxidant defense and cellular regulation. *JUBMB Life 50*, 301–307.

Li DZ and Pritchard HW (2009) The science and economics of *ex situ* plant conservation. *Trends in Plant Science* 14, 614–621.

Li L, Nelson CJ, Alexova R, Jacoby RP and Millar AH (2010) Protein degradation rate in *Arabidopsis thaliana* leaf growth and development. *Plant Cell* 29, 207–228.

Li SC, Chung MC and Chen CS (2001) Cloning and characterization of a DEAD box RNA helicase from the viable seedlings of aged mung bean. *Plant Molecular Biology* 47, 761–770.

Li T, Zhang Y, Wang D, Liu Y, Dirk LMA, Goodman J, Downie AW, Wang J, Wang G and Zhao T (2017b) Regulation of seed vigor by manipulation of raffinose family oligosaccharides (RFOs) in maize and Arabidopsis. *Molecular Plant* 10, 1540–1555.

Ling J and Soll D (2010) Severe oxidative stress induces protein mistranslation through impairment of an aminoacyl-tRNA synthetase editing site. *Proceedings of the National Academy of Sciences of the USA* 107, 4028–4033.

Liu Y, Zheng Y, Zhang Y, Wang W and Li R (2010) Soybean FM2 protein (LhK3) confers the tolerance of *Escherichia coli* and stabilization of enzyme activity under diverse stresses. *Current Microbiology* 50, 373–378.

Lloyd J and Meinke D (2012) A comprehensive dataset of genes with a loss-of-function mutant phenotype in Arabidopsis. *Plant Physiology* 158, 1115–1129.

Lorkovic ZJ, Herrmann RG and Oelmuller R (1997) PRH75, a new nucleus-localized member of the DEAD-box protein family from higher plants. *Molecular and Cellular Biology* 17, 2257–2265.

Maekawa S, Ishida T and Yanagisawa S (2018) Reduced expression of apn24, encoding a novel rna processing factor, induces sugar-dependent nucleolar stress and altered sugar responses in *Arabidopsis thaliana*. *Plant Journal* 30, 209–227.

Maillard M-L.-C. (1912) Action des acides amines sur les sucres; formation des melanoidines par voie methodique. *Academie des sciences des seances de l'Academie des sciences* 154, 66–68.

Manfre AJ, Lanni LM and Marcotte Jr W.R. (2006) The Arabidopsis group 1 LATE EMBRYOGENESIS ABUNDANT protein ATEM6 is required for normal seed development. *Plant Physiology* 140, 140–149.

Mettimmole V and Battista JR (1996) Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *Journal of Bacteriology* 178, 633–637.

Mauro VP and Edelman GM (2002) The ribosome filter hypothesis. *Proceedings of the National Academy of Sciences USA* 99, 12031–12036.

McLoughlin F, Basha E, Fowler ME, Kim M, Bordowicz J, Katyar-Agarwal S and Vierling E (2016) Class I and II small heat proteins reveal their relevance in the adaptive response during water deficit in *Arabidopsis thaliana*. *Plant Physiology* 172, 1221–1236.

Mene-Saffrane L, Jones AD and DellaPenna D (2010) Plastochromanol-8 and tocopherols are essential lipid-soluble antioxidants during seed desiccation and quiescence in Arabidopsis. *Proceedings of the National Academy of Sciences USA* 107, 17815–17820.

Menz MA, Boswell L, Toner M and Hand SC (2009) Occurrence of mitochondria-targeted Late Embryogenesis Abundant (LEA) gene in animals increases organelle resistance to water stress. *Journal of Biological Chemistry* 284, 10714–10719.

Meyer Y, Belin C, Delorme-Hinoux V, Reichheld JP and Riondet C (2012) Thioderoxin and glutaredoxin systems in plants: molecular mechanisms, crosstalks, and functional significance. *Antioxidant and Redox Signaling* 17, 1124–1160.

Min CW, Lee SH, Cheon YE, Han WY, Ko JM, Kang HW, Kim YC, Agrawal GK, Rakwal R, Gupta R and Kim ST (2017) In-depth proteomic analysis of *Glycine max* seeds during controlled deterioration treatment reveals a shift in seed metabolism. *Journal of Proteomics* 169, 125–135.

Moore JP, Le NT, Brandt WF, Dziouich A and Farrant JM (2009) Towards a systems-based understanding of plant desiccation tolerance. *Trends in Plant Science* 14, 110–117.

Mtwisha L, Brandt W, McCready S and Lindsey GG (1998) HSP 12 is a LEA-like protein in *Saccharomyces cerevisiae*. *Plant Molecular Biology* 37, 513–521.

Mudgett MB and Clarke S (1994) Hormonal and environmental responsiveness of a developmentally regulated protein L-isopalatitis methyltransferase in wheat. *Journal of Biological Chemistry* 269, 25605–25612.

Mudgett MB, Lowenson JD and Clarke S (1997) Protein repair L-isopalatitis methyltransferase in plants. Phylogenetic distribution and the accumulation of substrate proteins in aged barley seeds. *Plant Physiology* 115, 1481–1489.

Muslin EH and Homann PH (1992) Light as a hazard for the desiccation-resistant ‘Resurrection’ fern *Polypodium polypoideioides*. *Plant Cell and Environment* 15, 81–89.

Nakabayashi K, Okamoto M, Koshiba T, Kamiya Y and Nambara E (2005) Genome-wide profiling of stored mRNA in *Arabidopsis thaliana* seed germination: epigenetic and genetic regulation of transcription in seed. *Plant Journal* 41, 697–709.

Nambara E and Nonogaki H (2012) Seed biology in the 21st century: perspectives and new directions. *Plant and Cell Physiology* 53, 1–4.

Nayak NR, Putnam AA, Addepalli B, Lowenson JD, Chen T, Jankowsky E, Perry SE, Dinkins RD, Limbach PA, Clarke SG and Downie AB (2013) An Arabidopsis ATP-dependent, DEAD-box RNA helicase loses activity upon IsoAsp formation but is restored by PROTEIN ISOASPARTYL METHYLTRANSFERASE. *Plant Cell* 25, 2573–2586.

Nelson CJ, Alzovra R, Jacoby RP and Millar AH (2014) Proteins with high turnover rate in barley leaves estimated by proteome analysis combined with in planta isotope labeling. *Plant Physiology* 166, 91–108.

Netzer N, Goodenbour JM, David A, Dittmar KA, Jones RB, Schneider JR, Boone D, Eves EM, Rosner MR, Gibbs JS, Embry A, Dolan B, Das S, Hickman HD, Berglund P, Bennink JR, Yewdell JW and Pan T (2009) Innate immune and chemically triggered oxidative stress modifies translational fidelity. *Nature* 462, 522–526.

Nguyen TP, Cuffe G, Hegedus DD, Rajou I and Bentiski L (2015) A role for seed storage proteins in Arabidopsis seed longevity. *Journal of Experimental Botany* 66, 6399–6413.

Oge L, Bourdais G, Bove J, Collet B, Godin B, Granier F, Job D, Hickman HD, Berglund P, Bennink JR, Yewdell JW and Pan T (2009) Innate immune and chemically triggered oxidative stress modifies translational fidelity. *Nature* 462, 522–526.

Oliver MJ, Yilmaz H and Mishler BD (2000) The evolution of vegetative desiccation tolerance in land plants. *Plant Ecology* 151, 85–100.

Oliver MJ, Velten J and Mishler BD (2005) Desiccation tolerance in bryophytes: a reflection of the primitive strategy for plant survival in dehydrating habitats? *Integrative and Comparative Biology* 45, 788–799.

Olvera-Carrillo Y, Campos F, Reyes JL, Garcirriubio A and Covarrubias AA (2010) Functional analysis of the group 4 late embryogenesis abundant proteins reveals their relevance in the adaptive response during water deficit in Arabidopsis. *Plant Physiology* 154, 373–390.

Oracz K, El-Maarouf Bouteau H, Farrant JM, Cooper K, Belghazi M, Job C, Job D, Corbineau F and Bailey C (2007) ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. *Plant Journal* 50, 452–465.

Pereira Lima JJ, Buitink J, Lalanne D, Rossi RF, Pelletier S, da Silva EAA and Leroy E (2017) Molecular characterization of the acquisition of long-term seed maturation in soybean. *PLoS One* 12, e0180282.

Personat JM, Tejedor-Canjo C, Prieto-Dapena P, Almoguera C and Jordano I (2014) Co-overexpression of two Heat Shock Factors results in enhanced seed longevity and in synergistic effects on seedling tolerance to severe dehydration and oxidative stress. *BMC Plant Biology* 14, 56.

Picard D (2002) Heat-shock protein 90, a chaperone for folding and regulation. *Cellular and Molecular Life Sciences* 59, 1640–1648.

Potts M, Slaughter SM, Hunneke FU, Garst JF and Helm RF (2005) Desiccation tolerance of prokaryotes: application of principles to human cells. *Integrative and Comparative Biology* 45, 800–809.

Prieto-Dapena P, Castano R, Almoguera C and Jordano I (2006) Improved resistance to controlled deterioration in transgenic seeds. *Plant Physiology* 142, 1102–1112.

Rajjou L, Lovigny Y, Groot SP, Belghazi M, Job C and Job D (2008) Proteome-wide characterization of seed aging in Arabidopsis: a comparison
Van den Ende W (2013) Multifunctional fructans and raffinose oligosaccharides. *Frontiers in Plant Science* 4, 247.

Verma P, Kaur H, Petla BP, Rao V, Saxena SC and Majee M (2013) PROTEIN L-ISOASPARTYL METHYLTRANSFERASE2 is differentially expressed in chickpea and enhances seed vigor and longevity by reducing abnormal isoaspartyl accumulation predominantly in seed nuclear proteins. *Plant Physiology* 161, 1141–1157.

Vertucci CW (1989) Effects of cooling rate on seeds exposed to liquid nitrogen temperatures. *Plant Physiology* 90, 1478–1485.

Wang L, Ma H, Song L, Shu Y and Gu W (2012) Comparative proteomics analysis reveals the mechanism of pre-harvest seed deterioration of soybean under high temperature and humidity stress. *Journal of Proteomics* 75, 2109–2127.

Waszczak C, Akter S, Jacques S, Huang J, Messens J and Van Breusegem F (2015) Oxidative post-translational modifications of cysteine residues in plant signal transduction. *Journal of Proteomics* 75, 2109–2127.

Waterworth WM, Bray CM and West CE (2015) The importance of safeguarding genome integrity in germination and seed longevity. *Journal of Experimental Botany* 66, 2923–2934.

Xie C, Zhang R, Qu Y, Miao Z, Zhang Y, Shen X, Wang T and Dong J (2012) Overexpression of MtCAS31 enhances drought tolerance in transgenic Arabidopsis by reducing stomatal density. *New Phytologist* 195, 124–135.

Xu H, Wei Y, Zhu Y, Lian L, Xie H, Cai Q, Chen Q, Lin Z, Wang Z, Xie H and Zhang J (2015) Antisense suppression of LOX3 gene expression in rice endosperm enhances seed longevity. *Plant Biotechnol Journal* 13, 526–539.

Xu Q, Belcastro MP, Villa ST, Dinkins RD, Clarke SG and Downie AB (2004) A second protein L-isoaspartyl methyltransferase gene in Arabidopsis produces two transcripts whose products are sequestered in the nucleus. *Plant Physiology* 136, 2652–2664.

Zhang H, Luo M, Day RC, Talbot MJ, Ivanova A, Ashton AR, Chaudhury AM, Macknight RC, Hrmova M and Koltunow AM (2015) Developmentally regulated HEART STOPPER, a mitochondrially targeted L18 ribosomal protein gene, is required for cell division, differentiation, and seed development in Arabidopsis. *Journal of Experimental Botany* 66, 5867–5880.

Zhang X, Lu S, Jiang C, Wang Y, Lv B, Shen J and Ming F (2014) RbLEA, a late embryogenesis abundant protein gene isolated from *Rosa chinensis*, confers tolerance to *Escherichia coli* and *Arabidopsis thaliana* and stabilizes enzyme activity under diverse stresses. *Plant Molecular Biology* 85, 333–347.

Zhu JX, Doyle HA, Mamula MJ and Aswad DW (2006) Protein repair in the brain, proteomic analysis of endogenous substrates for protein L-isoaspartyl methyltransferase in mouse brain. *Journal of Biological Chemistry* 281, 33802–33813.