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Loïc Rajjou[*1,2], Manuel Duval[†1,3], Karine Gallardo[‡1,4], Julie Catusse[§1,5], Julia Bally[¶1,6], Claudette Job[‖1] and Dominique Job[**1]

[1]CNRS–Bayer CropScience Joint Laboratory, UMR 5240, Bayer CropScience, F-69263 Lyon Cedex 9, France
[2]Institut Jean-Pierre Bourgin, UMR 1318, INRA AgroParisTech, Saclay *Plant Sci*ences, F-78026 Versailles Cedex, France
[3]Network Therapeutics Foundation Inc., New London, Connecticut 06320
[4]INRA, UMR 102 LEG, F-21065 Dijon
[5]Quintiles, 67404 Illkirch, France
[6]School of Molecular Bioscience, University of Sydney, New South Wales 2006, Australia

Germination vigor is driven by the ability of the plant embryo, embedded within the seed, to resume its metabolic activity in a coordinated and sequential manner. Studies using "-omics" approaches support the finding that a main contributor of seed germination success is the quality of the messenger RNAs stored during embryo maturation on the mother plant. In addition, proteostasis and DNA integrity play a major role in the germination phenotype. Because of its pivotal role in cell metabolism and its close relationships with hormone signaling path-ways regulating seed germination, the sulfur amino acid metabolism pathway represents a key biochemical determinant of the commitment of the seed to initiate its development toward germination. This review highlights that germination vigor depends on multiple biochemical and molecular variables. Their characterization is expected to deliver new markers of seed quality that can be used in breeding programs and/or in biotechnological approaches to improve crop yields.

Introduction

Most flowering plants reproduce by sexual breeding and seed production. The success of seed germination and the establishment of a normal seedling are determining features for the propagation of plant species, which are of both economic and ecologic importance. Because of its high vulnerability to injury, disease, and water/environmental stress, germination is considered to be the most critical phase in the plant life cycle. By definition, this process incorporates events starting with the uptake of water by the mature dry seed and terminating with the protrusion of the radicle (or, more generally, a part of the embryo) through the seed envelopes. Thereafter, seedling growth is underway (figure 1). Germination is a complex process during which the imbibed mature seed must quickly shift from a maturationto a germination-driven program of development and prepare for seedling growth (109).

The seed results from double fertilization of the ovule by the pollen grain. It houses both a zygotic embryo that will form the new plant as well as a storage tissue to supply nutrients that support seedling growth following germination. This latter storage tissue is usually triploid (e.g., the endosperms of cereal grains); however, in some species the storage tissue may derive only from the maternal nucellus, such as the perisperm in sugar beet. In other angiosperm species, the embryo absorbs the endosperm as the latter grows within the developing seed, and the cotyledons of the embryo become filled with these storage compounds; at maturity, seeds of these species (e.g., pea, sunflower, and *Arabidopsis*) have only residual endosperm and are termed exalbuminous seeds.

Seeds fall into two broad categories: (a) the so-called orthodox seeds, which sustain intense desiccation by the end of their maturation on the mother plant and retain their germination potential over long periods of dry storage, and (b) the recalcitrant seeds, which do not survive drying during ex situ conservation (123). From an evolutionary perspective, seed desiccation tolerance seems associated with plants grown in drier environments, whereas desiccation sensitivity is most common in moist environments. Because of this unique property of survival during dry storage, orthodox seeds are the most commonly used in agriculture. However, economically important species such as avocado, mango, lychee, cocoa, coffee, citrus, and rubber produce recalcitrant seeds.

Here, we focus on the orthodox seeds for which desiccation during maturation allows metabolic activity to be interrupted and restarted during germination following a simple imbibition. In such seeds, preservation of embryonic cell viability in the dry state can extend over centuries (47, 131, 145). This implies the existence of specific mechanisms to maintain the state of metabolic quiescence in mature dry seeds while preserving their integrity to ensure that cell metabolism is activated and restarted during germination.

[*]loic.rajjou@agroparistech.fr
[†]manuel.duval@networktherapeutics.org
[‡]karine.gallardo@dijon.inra.fr
[§]julie.catusse@quintiles.com
[¶]julia.bally@sydney.edu.au
[‖]claudette.job@bayer.com
[**]dominique.job@bayer.com

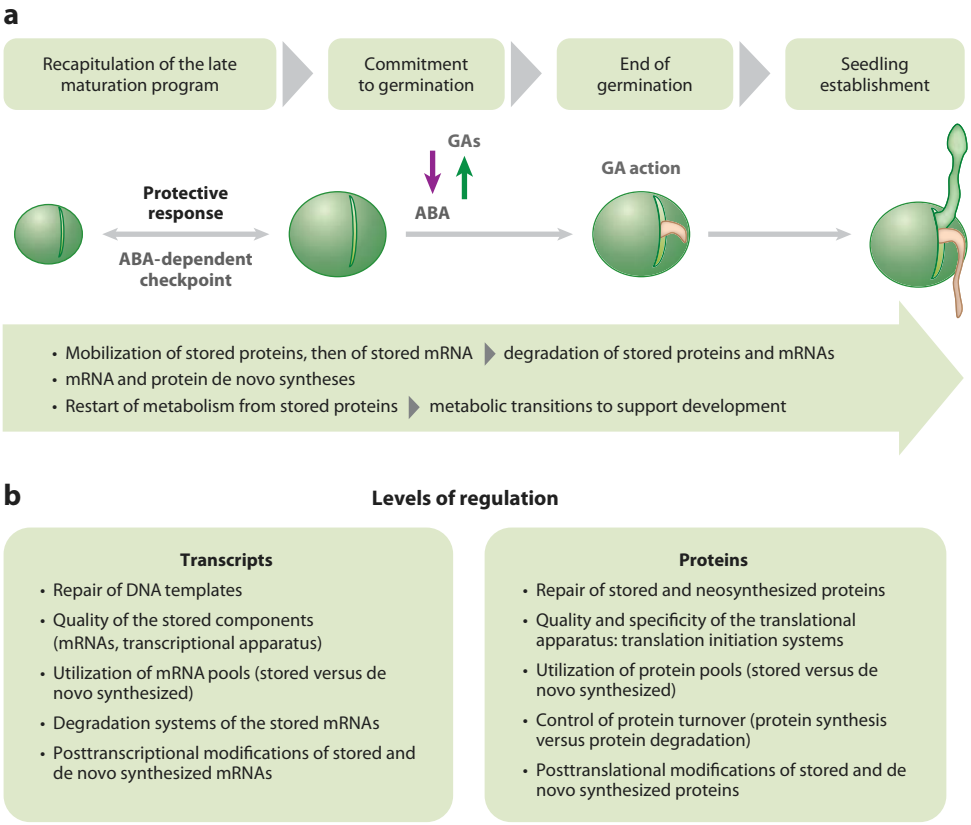

Figure 1: Importance of the stored and de novo synthesized messenger RNAs (mRNAs) and proteins in controlling seed germination. (a) Phenomenology of seed germination. This panel includes the three phases of water intake, germination sensu stricto, and radicle emergence, and highlights the need for stored and de novo synthesized components for germination to occur. (b) Levels of regulation of transcript and protein synthesis/ mobilization during germination. For example, germination relies on the quality of the components stored in mature seeds (e.g., transcription occurs from stored proteins) and on those components' ability to be utilized during germination (e.g., de novo synthesis of mRNA is not required for germination, but activates this process) or upon a stress [e.g., synthesis of late embryogenesis abundant (LEA) proteins from stored mRNAs, recapitulation of the late maturation program] (117). Germination also relies on the quality and specificity of the translational apparatus (e.g., the translation initiation factor eIfiso4E is particularly required for translation of the stored mRNAs; 40) and on posttranslational modifications such as carbonylation (74), phosphorylation (22, 63), and ubiquitination (10). Abbreviations: ABA, abscisic acid; GA, gibberellin.

The mature dry seeds of most species require a period of dry storage known as after-ripening to release them from dormancy (67), a physiological state in which seeds will not germinate even under optimal conditions that are otherwise favorable to their germination when they become nondormant (12).

For improved crop production, a main agricultural goal is to obtain rapid and uniform germination and seedling emergence once seeds are sown. Differences at these stages are generally not recovered, which directly impacts crop yield. To increase the performance of commercial seed lots, the seed industry practices invigoration treatments known as priming, which consist of a controlled imbibition of the seeds followed by dehydration back to their initial water content so as to permit their storage (21, 58, 98). These treatments are thought to somehow reproduce early stages of germination, suggesting that the primed seeds have an advance in germination time compared with untreated seeds (134).

Besides these technological solutions, recent work has emphasized the potential for genetically improving seed vigor. For example, quantitative trait loci (QTLs) related to germination rate have been detected in sunflower (3), *Brassica oleracea* (48) and *Medicago truncatula* (143). Quantitative genetics has also been applied to dissect the genetic architecture of seed germination under various environmental stresses, leading to the detection of QTLs controlling this trait (39, 55). fine mapping and cloning of these QTLs-as has been done with DOG1, a QTL controlling seed dormancy in *Arabidopsis* (18, 28, 76)-are shedding light on the molecular mechanisms governing the response of seed germination to environmental stress.

Aims of this review

Since 1900, there have been more than 25,000 publications on seed germination, reflecting both the agronomic interest and the complexity of this topic in plant sciences (109). With the advent of postgenomics technologies, high-throughput analyses of molecular profiles have been implemented at the RNA, protein, and metabolite levels to reveal specific features of the germination process. For example, the availability of these technologies led Carrera et al. (24) to generate a list of genes with differential expression in germinating seeds, referred to as the germination

signature, and a seedspecific gene ontology named TAGGIT to help in describing this signature. Similarly, the PageMan/MapMan package was used to visualize transcriptome changes in *Arabidopsis* (75) and barley (136) seeds during germination. Recently, a genome-wide network model named SeedNet, which describes transcriptional interactions during *Arabidopsis* seed germination, was also shown to accurately define regulators that behave as activators or inhibitors of germination (14). These findings thereby imply that the decision to remain dormant or enter into germination depends on the net activity of germination-promoting signals overcoming their inhibiting counterparts (14).

There are excellent reviews on many aspects of seed physiology in relation to germination, including dormancy, water relations, environmental factors, and hormonal control (42, 59, 67, 84, 147). Here, we first summarize the roles of some factors influencing seed germination, such as hormones, chemical stimulants, and repair processes. We then discuss phenomenological aspects of the germination process. finally, we focus on the current advances obtained through the postgenomics approaches, with special attention to three main aspects: (a) the translational control of germination and the role of stored components, (b) the importance of metabolic transitions in germination, and (c) the search for biomarkers of seed vigor.

Hormones and chemical stimulants in seed germination control

The phytohormone abscisic acid (ABA), a sesquiterpene compound resulting from the cleavage of carotenoids, controls storage reserve accumulation and desiccation tolerance of orthodox seeds (106). Notably, this hormone induces the expression of late embryogenesis abundant (LEA) proteins, which become abundant during late maturation and are thought to act as chaperones to protect macromolecular structures against desiccation injury (142). Importantly, during seed maturation, ABA also exerts an inhibitory effect on mechanisms triggering precocious and deleterious germination of developing seeds on the mother plant (preharvest sprouting), thereby allowing the maturation process to be sustained and seeds to be formed that are endowed with appropriate reserves required for establishment of vigorous plantlets following germination.

Therefore, to successfully germinate following imbibition and to counteract the inhibitory effect of ABA on germination, a nondormant seed must (a) establish a specific catabolism, decrease in sensitivity, and biosynthesis inhibition to reduce the active level of this hormone (4), and (b) synthesize another class of hormones represented by a large family of tetracyclic diterpenes, the gibberellins (GAs), which are essential germination activators (56, 81, 139) (figure 1). GAs negatively regulate proteins behaving as repressors of germination, such as the GRAS transcriptional regulators involved in cellular differentiation (113, 154). Consistent with the idea that the ABA/GA ratio regulates the metabolic transition required for germination (106, 151), imbibed dormant seeds retain high levels of bioactive ABA (4).

Other hormones-such as ethylene (77, 89, 137), brassinosteroids (31, 84), salicylic acid (86, 118), cytokinin (30, 99, 137), auxin (17, 84), jasmonic acid (115), and oxylipins (36)-also influence germination. All form an interlocked signaling network and interact with one another to finely control germination, particularly in response to environmental constraints.

Besides these phytohormones, a number of diverse germination stimulants are known, some of which are presumably important as environmental sensors. Among them, several nitrogen-containing compounds stimulate germination-including nitric oxide (NO) gas, nitrite (NO2), and nitrate (NO3-)- presumably via conversion into NO (2, 19). Also, reactive oxygen species (ROS) exert control over germination, most likely in concert with NO , to regulate ABA catabolism and GA biosynthesis during seed imbibition (90). Recently, karrikins have been established as a new class of signaling molecules in smoke from burning vegetation that trigger seed germination in many angiosperms, even for nonfire-prone species such as *Arabidopsis* (108). Related compounds such as strigolactones are germination stimulants produced by plant roots for seeds of the root parasitic weeds of the Orobanchaceae family (152).

Importance of cellular repair in germination and vigor

Dehydration and rehydration during seed development and germination are associated with high levels of oxidative stress, resulting in DNA damage (34). Genome damage also occurs during seed storage, leading to a loss of seed vigor and viability (27). Damaged DNA must therefore be repaired during germination (figure 1). An essential step is the DNA ligase-mediated rejoining of singleand double-strand breaks. In *Arabidopsis*, inactivation of the plant-specific DNA LIGASE VI entails a delay in seed germination (146). The atlig6 mutants also display hypersensitivity to seed aging, thus implicating AtLIG6 as a major determinant of *Arabidopsis* seed quality and longevity (146). That DNA repair is required for seed germination is also supported by the observation that enzymes involved in the repair of oxidatively damaged DNA (e.g., formamidopyrimidine-DNA glycosylase and 8-oxoguanine DNA glycosylase/ lyase) are upregulated during early stages of *Medicago truncatula* seed germination (96).

Because nicotinamide is an inhibitor of poly(ADP-ribose)polymerases(PARPs),which are implicated in DNA repair, this compound must be degraded during germination. An *Arabidopsis* gene, NIC2, encoding a nicotinamidase enzyme is expressed at relatively high levels in mature seeds (65). Seeds of a knockout mutant, *nic2-1*, show reduced

nicotinamidase activity and retarded germination, suggesting that nicotinamide is normally metabolized by NIC2, which relieves inhibition of PARP activity and allows DNA repair to occur (65).

Seed proteins are also subject to aging injury (figure 1). Protein L-isoaspartylO-methyltransferase (PIMT) functions to initiate the repair of isomerized aspartyl and asparaginyl residues that spontaneously accumulate with age in proteins of a variety of organisms. In *Arabidopsis*, a decreased accumulation of the repair methyltransferase entails decreased seed longevity and germination. The opposite was observed following overexpression of this enzyme (110). Therefore, it is likely that the PIMT repair pathway allows the active elimination of deleterious protein products during germination and seedling establishment (110). Seeds from most species exhibit decreased PIMT activity during germination; however, the sacred lotus seeds, which have a proven record of exceptional longevity, display a persistent activity of the PIMT enzyme during germination, associated with a minimal degree of aspartyl racemization in proteins (131).

Phenomenology of seed germination

Seed germination is traditionally described as corresponding to a process comprising three phases, starting with water intake during which the seed imbibes (phase 1) and reinitiates metabolic processes (phase 2) and followed by the emergence of the radicle through the seed envelopes (phase 3) (figure 1) (109).

Reactivation of cellular activity during germination

Phase 1 does not simply correspond to physical seed imbibition, as changes in gene expression have been detected in *Arabidopsis* seeds as soon as 15 min after imbibition (115). Similarly, in a study of rice seeds, rapid changes in some metabolite levels had already occurred at 1h after imbibition (HAI) (62).

The maturation program can be recapitulated during early stages of germination

Several studies have established that the development of a germinating embryo into an autotrophic seedling could be arrested under conditions of water deficit. This growth arrest corresponds to an ABA-dependent checkpoint of germination involving the ABI5 transcription factor (92, 93). Genetic analyses disclosed that the embryogenic ABI3 factor, which acts upstream of ABI5 and is essential for the expression of ABI5, is also required to promote this growth arrest. Because the activity of ABI3 is essential at the end of the maturation phase, its accumulation during the early stages of germination in the presence of ABA or an osmotic stress reflects a reinduction of developmental processes normally taking place at the end of maturation. In other words, this initial phase of germination is a reversible process in which the maturation program can be recapitulated (figure 1). Based on the known function of the genes controlled by ABI3 during seed maturation, notably those encoding LEA proteins, Lopez-Molina et al. (92) proposed that the formation of embryos arrested in their progress toward germination corresponds to an adaptive mechanism that increases the survival of seeds under conditions of water stress that may be encountered in the soil.

Proteomics has confirmed that imbibed *Arabidopsis* seeds can reset the maturation program (117, 118). The possibility of recapitulating the late maturation program at two levels- the transcriptional (93) and the translational (117, 118)-would allow rapid adjustment of the response of imbibed seeds confronted with rapid fluctuations of environmental conditions, e.g., water availability in the soil.

The timing of gibberellin action during seed germination

A proteomic study of seeds from the GAdeficient *Arabidopsis* ga1 mutant indicated a rather late implication of GAs in germination, occurring at a stage coinciding with or very close to radicle emergence (figure 1) (54). It appears that GAs, although required for the completion of germination, are not directly involved in many processes taking place during germination, such as the initial mobilization of seed storage proteins and lipids. Out of 46 protein changes detected during germination (24 HAI), only 1, corresponding to the cytoskeleton component α-2,4-tubulin, appears to depend on the action of GAs (54). This implies that imbibed ga1 mutant seeds are metabolically active, although they will not complete germination (54). This behavior was confirmed by a study of *Arabidopsis* seed germination combining measurements of endogenous GA levels by gas chromatography–mass spectrometry and transcriptomic analyses of GA-regulated genes (111).

A last step in *Arabidopsis* seed germination is linked to oxylipin metabolism

In *Arabidopsis*, *COMATOSE* (CTS)-a singlecopy gene encoding an ATP-binding cassette (ABC) carrier involved in the mobilization of reserve lipids-is required for seed germination (24). The mutant *cts* seeds accumulate large amounts of oxylipins (a family of oxygenated products formed from fatty acids) during late maturation that behave as strong germination inhibitors (36). Because their protein profile resembles that of dormant seeds, the mutant seeds have been described as being forever dormant (125). However, contrary to expectation, the transcriptomic profile of the imbibed *cts* seeds closely resembles that of imbibed nondormant seeds (24). In other words, the *cts* mutant seeds

can manage to leave their dormant state, although they are unable to germinate. This has led to the assumption that *CTS* has a direct role in germination just before the emergence of the radicle, rather than inhibiting dormancy release during after-ripening (24). A comparison of the transcriptome of imbibed *cts* mutant seeds with that of wild-type nondormant seeds incubated in the presence of GAs (111) showed that the function of *CTS* is required after the establishment of the GA mode of action during germination (24).

Translational and posttranslational control of seed germination

Translation initiation and role of stored messenger RNAs

Translational processes play a pivotal role during seed germination (35, 43, 60, 97, 117). In particular, a characteristic mechanism of gene expression regulation during germination is the selective translation of messenger RNAs (mRNAs) (figure 1). This is of importance, as a large percentage of the genome's encoded mRNAs are present in dry *Arabidopsis* seeds (105). Several studies have documented the role of these stored mRNAs in germination.

The translational activities of the translation initiation factors eIF4E and eIfiso4E were tested in vitro using transcripts from dry and 24-h-imbibed maize embryonic axes (40). The data indicated that eIfiso4E is particularly required for translation of the stored mRNAs from dry seeds, and that eIF4E is unable to fully replace the eIfiso4E activity. Besides the small cap-binding subunit (eIF4E), the eukaryotic translation initiation cap-binding complex eIF4F contains a larger protein subunit (eIF4G) that facilitates interactions with other initiation factors. *Arabidopsis* knockout lines for the plant-specific eukaryotic translation initiation factor genes *i4g1* and *i4g2*, which encode two isoforms of the eIF4G family (eIfiso4G1 and eIfiso4G2), have been obtained. Double-mutant *i4g1/i4g2* knockout plants show reduced germination rates, slow growth rates, and reduced seed viability (87).

At some points of a cell's life-for example, during cell stress-the standard mechanism of translation initiation via cap recognition at the 5' end of cellular mRNAs is compromised. In those cases, some mRNAs use an alternative, cap-independent form of initiation as a congregation site for initiation factors (11). A proteomic analysis of sugar beet seeds revealed the possibility of initiating translation through either the cap-dependent mechanism or this cap-independent process (26). Consistent with this, some stored mRNAs are efficiently translated via a cap-independent mechanism during maize embryonic axes germination (41).

Confirming previous results (43), another study (117) used metabolic inhibitors to show that de novo transcription is not required for completion of germination, as inferred from the observation that radicle protrusion through the seed coats can occur in *Arabidopsis* following seed imbibition in the presence of α-amanitin, a highly potent and specific inhibitor of DNA-dependent RNA polymerase II. The speed and uniformity of germination are nonetheless strongly affected under these conditions, implying that transcripts must be synthesized de novo during germination to increase germination vigor, notably those encoding enzymes and proteins required for the biosynthesis of GAs and/or those involved in the sensitivity of germination to this hormone (figure 1). Also, seeds germinated in the presence of α-amanitin seem to recapitulate at least part of the seed maturation program, as increased accumulation of 12S globulin subunits and members of the dehydrin family (group 2 LEA proteins) has been observed (117). Thus, commitment to germination requires transcription of genes allowing the imbibed seed to discriminate between those mRNAs that will be utilized in germination and those that will be destroyed (figure 1). In contrast, the germination of *Arabidopsis* seeds is entirely blocked in the presence of cycloheximide, a translation inhibitor (117). A study of rice seed germination led to similar findings (57).

Altogether, these results provide evidence that protein synthesis is required for the completion of germination, particularly from the stored mRNA templates, whose stability conditions seed vigor (129, 132). In agreement with earlier work (43, 129, 132, 140), these results highlight the role of mRNAs and proteins stored in seeds during their maturation on the mother plant and show that in *Arabidopsis*, the potential for seed germination is largely programmed during the maturation process. Interestingly, the classes of abundant stored mRNAs are highly conserved in mature barley and *Arabidopsis* seeds. Because monocot-dicot divergence occurred approximately 200 Mya, such conservation of stored mRNAs in both monocot and dicot dry seeds reinforces the hypothesis that those transcripts and the pathways they encode are functionally important to seed germination in all species (6).

It is well established that seeds contain functional DNA-dependent RNA polymerases (71). The function of the seed-stored transcriptional machinery in the resumption of gene expression after the onset of seed imbibition has been investigated in *Arabidopsis*. Changes in the mRNA abundance of many genes are initiated as soon as between 1 and 2 HAI, and microarray and reverse transcription polymerase chain reaction (RT-PCR) expression analysis of imbibition-responsive genes indicated that this early induction is not altered by treatment with cycloheximide, although this molecule blocked germination at later stages (radicle protrusion) (79). Thus, in contrast to what occurs at the end of germination (79, 117), de novo protein synthesis is not required for gene expression during early imbibition stages, and seed-stored components of the transcriptional machinery are sufficient (79) (figure 1). A comparison of the results of Rajjou et al. (117) (radicle protrusion) with those of Kimura & Nambara (79) (early imbibition) reveals the roles of the protein and mRNA pools stored in the mature dry seeds at successive stages of the germination process. It appears that very early, the stock of stored proteins is used to restart cellular activity, which is followed by mobilization of the stored mRNA pool (figure 1). This role of the stored proteins provides additional evidence to support the concept that germination is prepared during maturation. Similar findings were reported for barley seed germination (136).

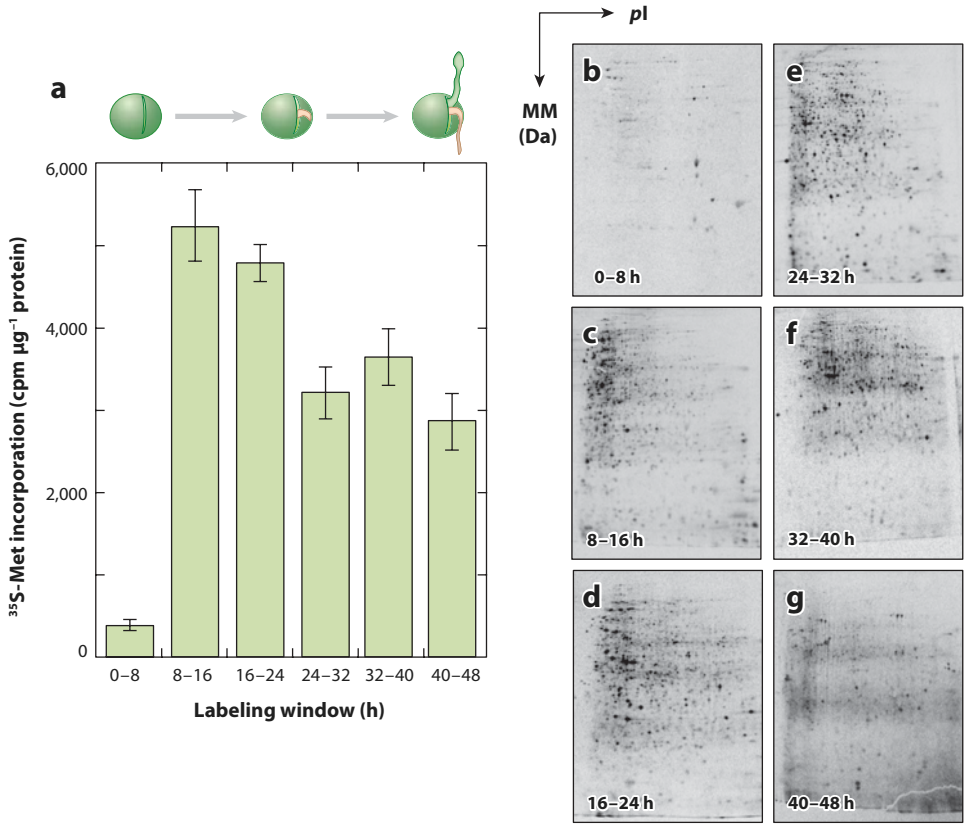

Figure 2: Dynamic proteomics during *Arabidopsis* seed germination. *Arabidopsis* seed proteins are radiolabeled in the presence of ^{35}S-methionine (^{35}S-Met) added to the germination medium during the labeling periods of 0–8 h, 8–16 h, 16–24 h, 24–32 h, 32–40 h, or 40–48 h. Total proteins are extracted and submitted to two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), and the radioactive proteins are revealed by autoradiography. (a) Total incorporation of ^{35}S-Met into proteins (cpm, counts per minute). (b–g) 2D protein profiles of de novo synthesized proteins during germination and seedling establishment. Abbreviations: MM, molecular mass (in daltons); pI, isoelectric point (pH at which a particular protein carries no net electrical charge). figure data adapted from Reference 118 and L. Rajjou, R. Huguet, C. Job & D. Job, unpublished data.

The progressive buildup of the *Arabidopsis* proteome during germination is well illustrated by a kinetic study of the newly synthesized proteins in which pulses of ^{35}S-methionine (^{35}S-Met) were provided to the seeds at various times during germination (118). Consistent with the results of Kimura & Nambara (79), translational activity is low during the first 8 h of imbibition, reflecting the use of stored proteins in this early phase (figure 2) (118). Translational activity then strongly increases (by more than tenfold) to reach a maximum during the labeling periods of 8–16h and 16–24h (figure 2) (118).

A comprehensive profile of the transcriptome and metabolites during germination in the monocot model rice disclosed a series of temporal switches in metabolites and transcripts accounting for a reactivation of cellular metabolism to support growth (62). At the earliest time point analyzed in this study (1 HAI), there were a greater proportion of detected metabolites than detected transcripts changing in abundance. These early responses were followed by a large change in transcript abundances between 3 and 12 HAI, and then by relatively small changes in transcripts at subsequent time points. In contrast, changes in a large number of metabolites continued up to 48 HAI. This behavior suggests that the early changes in metabolites arise from the activity of preexisting enzymes, consistent with the *Arabidopsis* features discussed above, whereas the later changes in metabolites are more likely driven by transcription and translation (62).

In sunflower, alleviation of seed dormancy during after-ripening is associated with an oxidation of stored mRNAs, thus altering their translation. This nonenzymatic oxidation is not random but selective, and targets transcripts previously identified as putative players in seed dormancy (16). It will be interesting to investigate whether this mechanism also operates during germination. It is noted that the degradation of a specific subset of mRNAs is a prerequisite to germination (62, 150).

Control of seed germination via posttranslational modifications

Proteomic investigations have highlighted that seed proteins are subjected to a large number of posttranslational modifications (PTMs), which may affect protein functions including localization, complex formation, stability, and activity (reviewed in 10) (figure 1).

Redox signaling Germination is accompanied by extensive change in the redox state of seed proteins. In cereals, proteins of both the starchy endosperm and embryo that are present mainly in the oxidized (S-S) form in the dry seed are converted to the reduced or sulfhydryl (-SH) state following imbibition (23). A regulatory disulfide protein, thioredoxin (Trx), plays a central role in this redox conversion. When reduced enzymatically in the presence of nicotinamide adenine dinucleotide phosphate (NADPH), Trx acts as a signal early in germination to facilitate the mobilization of reserves by (a) reducing storage proteins, thereby enhancing their solubility and susceptibility to proteolysis; (b) reducing and inactivating disulfide proteins that inhibit specific amylases and proteases, thereby facilitating the breakdown of stored starch and proteins; and (c) reductively activating individual enzymes functional in germination (23). Similar observations have been made during germination of seeds of the model legume *Medicago truncatula*, showing that Trx functions in the germination of seeds of dicotyledons as described in monocotyledons (5).

These studies suggest a mechanism in which the oxidized form of the proteome in mature dry seeds accounts for metabolic quiescence, and suggest that this proteome is activated during germination by a reduction of protein disulfide bridges in the presence of Trx (23). This is equivalent to the role of Trx in the reversible light activation of key photosynthetic enzymes in the Calvin-Benson cycle (130).

Interestingly, FK506-binding proteins (FKBPs) and cyclophilins are redox regulated by Trx in plants (103). FKBPs and cyclophilins have been found in Trx screens of germinating seeds (5). These proteins belong to the peptidyl-prolyl *cis-trans* isomerase family catalyzing the interconversion of peptidyl-prolyl imide bonds in peptide and protein substrates. Thus, the *cis/trans* isomerization of the peptide bond during seed germination is emerging as a basic molecular switch that may modulate protein function.

Another oxidative modification of proteins results from protein carbonylation by ROS, a process known to contribute to various diseases in humans, including aging, Alzheimer's Disease, Parkinson's Disease, cancer, and heart diseases. Various carbonylated proteins accumulate during imbibition of *Arabidopsis* seeds (74). This process targets specific metabolic enzymes, translation factors, and several molecular chaperones. Although accumulation of carbonylated proteins is usually considered in the context of aging in a variety of model systems, this is clearly not the case in seeds, because *Arabidopsis* seeds-despite containing large quantities of carbonylated proteins-can germinate at a high rate and yield vigorous plantlets.

It appears that the observed specific changes in protein carbonylation patterns are probably required for counteracting and/or utilizing the production of ROS caused by recovery of metabolic activity in the germinating seeds (83). Notably, the observed carbonylation of highly abundant seed storage proteins could reflect their role in scavenging the ROS produced during seed germination (74). Also, it has been proposed that this could facilitate the mobilization of the seed storage proteins during seedling establishment, presumably by destabilizing their structure and promoting proteolytic attack (74).

Phosphorylation/dephosphorylation Phosphorylation and dephosphorylation represent ubiquitous regulatory mechanisms in cell signaling. A set of protein phosphatases and protein kinases has been shown to be involved in the control of germination through the modulation of ABA signaling (22, 63). Also, it has been proposed that phosphorylation of enzymes involved in DNA repair (including the mismatch binding protein Mus3 as well as PARP I; 95) and protein translation (initiation factors and ribosomal proteins; 85, 100) are crucial to allowing proper molecular control of seed germination.

Nitric oxide–mediated posttranslational modifications NO° is a major and versatile mediator in biological systems. However, although NO° has been shown to modulate metabolic activity in seeds, its mode of action in the control of germination remains undocumented. It has been shown to modulate protein function-byPTMs;indeed,itcanbindtotransition metals of metalloproteins (metal nitrosylation) or cause cysteine (Cys) *S*-nitrosylation or tyrosine nitration (10, 101). A transient burst of NO° was observed during the first hours of *Arabidopsis* seed imbibition (90); furthermore, an increment of several nitrated proteins was observed in sorghum embryonic axes at 24HAI (70). Protein nitration would be more than abiological marker of nitrosative stress and could participate in protein turnover or signal transduction in plants (32, 68).

Interestingly, tyrosine nitration of the molybdenum cofactor sulfurase (ABA3), an enzyme involved in the last step of ABA synthesis, has been reported in *Arabidopsis* (94). An inactivation of ABA synthesis by this mechanism during seed imbibition might contribute to determining the potential for germination. Moreover, growing evidence suggests that *S*-nitrosylation of proteins may regulate metabolic and energetic processes involved in seed germination (10). Because of its selectivity toward protein targets, this PTM may represent a prevalent pathway for modulating protein structure and function, comparable to protein phosphorylation (135).

Other posttranslational modifications Among more than 300 indexed protein chemical modifications, and beyond the PTMs mentioned above, the contributions of protein biotinylation, glycosylation, ubiquitination, farnesylation, and acetylation in germination have also been experimentally demonstrated (reviewed in 10). Thanks to technological advancements in high-throughput assays for PTM determination and more intensive basic research in this field, seed scientists are assuredly at the dawn of a new age in understanding the roles of specific PTMs and their protein targets in germination vigor (figure 1).

Metabolic transitions in seed germination

The role of metabolism in seed development and germination was recently revisited by the use of large-scale metabolomics approaches. Fait et al. (46) showed that in *Arabidopsis*, the transition from reserve accumulation to seed desiccation is associated with a major metabolic switch, resulting in the accumulation of distinct sugars, organic acids, nitrogen-rich amino acids, and shikimate-derived metabolites. In contrast, seed stratification (an imbibition of dormant or nondormant seeds at low temperature, which encourages subsequent germination and can be viewed as a priming treatment) is associated with a decrease in the content of several of these metabolic intermediates, implying that they might support the metabolic reorganization needed for seed germination. Concomitantly, the levels of other metabolites significantly increase during stratification and are boosted further during germination, implying their importance for germination and seedling establishment. Interestingly, a significant proportion of the gene expression and metabolic signatures of seed desiccation resemble those characterizing seed germination, implying that the preparation of the seeds for germination begins during seed desiccation (8, 46).

Met metabolism is central to seed germination

Among the essential amino acids synthesized by plants, Met is a fundamental metabolite because it functions not only as a building block for protein synthesis but also as the precursor of *S*-adenosylmethionine (AdoMet), the universal methyl-group donor, and as the precursor of polyamines, the plant-ripening hormone ethylene, and the vitamin biotin (121, 141). In plants, Met can be synthesized through two pathways (figure 3). In the de novo biosynthetic pathway, O-phosphohomoserine (OPH) is transformed first to cystathionine (Cyst) in a reaction catalyzed by Cyst γ-synthase, then to homocysteine (Hcy) in a reaction catalyzed by Cyst β-lyase, and finally to Met in the presence of the cobalamin-independent Met synthase (121, 122). In the Met recycling pathway, *S*-methylmethionine (SMM), a compound unique to plants, is synthesized by a methyl transfer from AdoMet to Met in a reaction catalyzed by AdoMet:Met Smethyltransferase. SMM can then be reconverted to Met by transferring a methyl group to Hcy in a reaction catalyzed by SMM:Hcy *S*-methyltransferase. These reactions, together with the reactions catalyzed by AdoMet synthetase and *S*-adenosylhomocysteine (AdoHcy) hydrolase, constitute the SMM cycle, which may be the main mechanism in plants for shortterm control of AdoMet level (120). Furthermore, in plants, AdoMet is an effector in the posttranscriptional autoregulation of the Cyst γ-synthase gene (29) and an allosteric regulator of threonine (Thr) synthase (33) (figure 3). Met metabolism is a housekeeping mechanism in all organisms. Here, we highlight the specific features of this mechanism in relation to seed germination.

During *Arabidopsis* seed germination, enzymes in this pathway show differential expression. Thus, the accumulation level of Met synthase increases strongly at 24 HAI, prior to radicle emergence. Its level is not increased further at 48 HAI, coincident with radicle emergence. Another enzyme corresponding to AdoMet synthetase specifically accumulates at the moment of radicle protrusion (52–54). Consistent with a role of the Met biosynthesis pathway in germination, DL-propargylglycine- a specific inhibitor of Met synthesis-delays seed germination and blocks seedling growth in *Arabidopsis*. Furthermore, these phenotypic effects are substantially alleviated upon Met supplementation in the germination medium (53).

Additional evidence for the role of this Met cycle in germination is provided by the use of other chemicals targeting this pathway. Thus, 9-(S)-(2,3-dihydroxypropyl)-adenine, an inhibitor of AdoHcy hydrolase, strongly delays tobacco seed germination and seedling growth (50), presumably by impeding Met recycling (figure 3) and methylation reactions, because AdoHcy is a highly potent competitive inhibitor of AdoMet-dependent methyltransferases. Also, methotrexate and aminopterin behave as potent inhibitors of *Arabidopsis* seed germination (13). These molecules are folic acid analogs that competitively inhibit dihydrofolate reductase, which is involved in folate biosynthesis. Folates function as one-carbon donors and play a critical role in thymidine and purine synthesis as well as in Met synthesis (figure 3). In dry pea seeds, the folate pool is present in very low concentration and increases considerably during germination and seedling establishment (69).

There exist several links between this Met metabolism and the control of germination.

Cysteine Cys is a precursor of Met biosynthesis (121) (figure 3) and constitutes a building block contributing to protein structure through the formation or reduction of disulfide bonds as catalyzed by Trxs. It is well documented that these enzymes affect a myriad of proteins during germination (23; see above and figure 3). Cys is also the precursor of the major antioxidant molecule glutathione (GSH), which is involved in several processes playing a role in germination-for example, the GSHascorbate cycle (20) or the formation of Snitrosoglutathione (GSNO), a storage form of NO that plays a pivotal role in seed physiology (19) (figure 3).

AdoMet-dependent methyltransferases There are a myriad of transmethylation reactions in plant cells, each of which is catalyzed by a specific AdoMet-dependent methyltransferase. Among these are the repair methyltransferase PIMT and the DNA methyltransferases that affect chromatin structure. It is well known that seed germination is associated with modifications of DNA methylation patterns (78, 105) and chromatin remodeling (153) (figure 3).

Other AdoMet-dependent methyltransferases influence hormone signaling (figure 3). first, magnesium chelatase and AdoMet:magnesium-protoporphyrin IX Omethyltransferase (BchM) catalyze sequential steps of the chlorophyll biosynthetic pathway. This methyltransferase forms a tight complex with magnesium-chelatase subunit H (127),

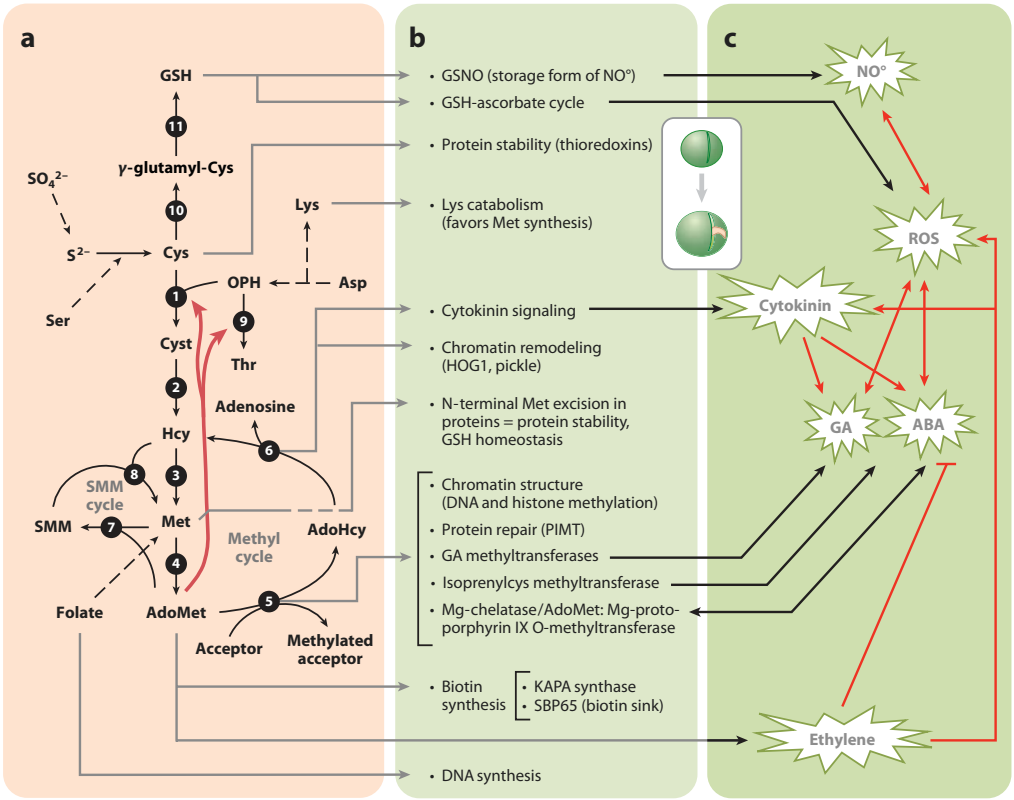

Figure 3: Sulfur amino acid metabolism in the control of seed germination. (a) Sulfur amino acid metabolism. O-phosphohomoserine (OPH) is a branch point intermediate between methionine/*S*-adenosylmethionine (Met/AdoMet) and threonine/isoleucine (Thr/Ileu) pathways. Enzymes: (step 1) cystathionine (Cyst) γ-synthase, (step 2) Cyst γ-lyase, (step 3) Met synthase, (step 4) AdoMet synthetase, (step 5) AdoMet-dependent transmethylases, (step 6) *S*-adenosylhomocysteine (AdoHcy) hydrolase, (step 7) AdoMet:Met *S*-methyltransferase, (step 8) *S*-methylmethionine:homocysteine (SMM:Hcy) *S*-methyltransferase, (step 9) Thr synthase, (step 10) γ-glutamyl-cysteine (γ-glutamyl-Cys) synthase, (step 11) glutathione (GSH) synthase. Dashed arrows indicate multiple reactions. The two red arrows indicate that AdoMet is an allosteric activator of Thr synthase (enzyme 9), allowing control over the partition of OPH fluxes between Met and Thr biosyntheses, and a regulator of Cyst γ-synthase (enzyme 1) gene expression. Between panels, different arrow colors are used to differentiate information transfer from internal metabolites of the sulfur amino acid pathway to hormonal signaling pathways: Gray denotes information transfer from internal metabolites (a) to biochemical pathways playing a role in seed germination (b); black denotes information transfer from biochemical pathways (b) to hormones or chemical stimulants of seed germination (c); and red denotes interconnections between hormone and chemical stimulant signaling pathways involved in seed germination control. Other abbreviations: Asp, aspartic acid; Lys, lysine. (b) Regulated processes. Abbreviations: GSNO, *S*-nitrosoglutathione; KAPA, 7-keto-8-aminopelargonic acid; PIMT, protein L-isoaspartyl-O-methyltransferase. (c) Links with hormones and signaling molecules. Abbreviations: ABA, abscisic acid; GA, gibberellin; ROS, reactive oxygen species.

which is an ABA receptor (148). Mutations in each of these genes are associated with seed germination and seedling establishment phenotypes (114, 148).

Second, a group of structurally and phylogenetically related AdoMet-dependent methyltransferases, called the SABATH family, was also identified in plants, notably during *Arabidopsis* seed maturation and germination (149). Biochemically characterized members of this family methylate the nitrogen atom or carboxyl groups found in a variety of plant hormones, thereby affecting their homeostasis in plant tissues. These hormones include salicylic acid, jasmonic acid, indole-3-acetic acid, and GAs (133, 144, 149, 155), all of which are involved in regulation of seed germination (see above).

Third, isoprenylated proteins bear an isoprenylcysteine methyl ester at the C-terminus. This is an important PTM of proteins in eukaryotic cells, as it facilitates protein– membrane and protein–protein interactions. *Arabidopsis* plants overexpressing isoprenylcysteine methyltransferase or isoprenylcysteine methylesterase exhibit marked changes in ABA sensitivity of seed germination, establishing these enzymes as potent regulators of ABA signaling (64).

AdoHcy hydrolase AdoHcy is a highly potent inhibitor of AdoMet-dependent methyltransferases. Therefore, the functioning of the methyl cycle is facilitated by the participation of AdoHcy hydrolase, which prevents accumulation of toxic levels of this compound. This enzyme plays a role in chromatin remodeling (e.g., HOG1, which is required for DNA methylation-dependent gene silencing, or the CHD3 remodeler PICKLE, which promotes trimethylation of histone H3), a process that regulates seed germination (124, 153). An AdoHcy hydrolase binds cytokinin with an enormous affinity; by doing so, this protein interferes with this hormonal signaling, leading to alteration in the ABA/GA balance (88). These results provide a link between cytokinins, DNA methylation, and germination (figure 3).

N-terminal Met excision in proteins The removal of the N-terminal Met in most cellular proteins is an important cotranslational protein modification catalyzed by Met aminopeptidases (figure 3). This process affects protein stability, and its inhibition is lethal in all organisms (49). Its partial inhibition strongly compromises seedling establishment in *Arabidopsis*, consistent with the finding that the N-end rule pathway, which targets protein degradation through the identity of the aminoterminal residue of specific protein substrates, promotes seed germination and seedling establishment (61). Interestingly, this phenotype is very similar to that of knockout mutants of γ-glutamyl-Cys synthetase, which is the first dedicated enzyme of the GSH biosynthesis pathway (49). Consistent with this, complementation assays reveal that the activity of GSH reductase [an enzyme controlling the ratio of oxidized GSH (GSSG) to GSH] is affected upon inhibition of N-terminal Met excision in proteins. Moreover, GSH or NADPH-the two substrates of this enzyme-could fully reverse the phenotypic defects (49).

Biotin AdoMet is a precursor of biotin, the cofactor of the biotin-dependent carboxylases, of which one, corresponding to acetyl–coenzyme A (CoA) carboxylase, is well known owing to its pivotal role in lipid synthesis (1). That biotin is needed for germination is shown by the observation that triphenyltin acetate, a very specific inhibitor of 7-keto-8-aminopelargonic (KAPA) acid synthase (an enzyme in the early step of the biotin biosynthesis pathway), blocks germination (66). This block in germination is reversed in the presence of biotin (66) (figure 3).

Besides the biotin-dependent carboxylases, plants contain a seed-specific biotinylated protein of approximately 65 kDa (hence named SBP65), which is devoid of carboxylase activity, covalently binds biotin at an atypical site compared with the consensus site in the biotin-dependent carboxylases, and belongs to the LEA protein group (44, 45, 73). As for LEA proteins, this seed-specific biotinylated protein accumulates in the late stages of seed maturation and disappears rapidly during seed germination. In contrast to humans and animals, plants do not express a biotinidase activity required for biotin recycling from biotinylated proteins (1). Therefore, altogether these findings have led to the hypothesis that this seed-specific biotinylated protein could function as a sink for biotin and hence as a repressor of metabolism in quiescent seeds, owing to the pivotal metabolic role of biotin in all organisms-i.e., it could function similarly to the avidin protein, a bacterial growth inhibitor that binds biotin with high specificity and affinity in raw eggs (1, 44) (figure 3).

Lysine metabolism Both lysine (Lys) and Met belong to the aspartic acid (Asp)–derived biosynthetic pathway, and the syntheses of these two essential amino acids are tightly interlocked (figure 3). Essential amino acids cannot be synthesized by humans or monogastric animals, which are dependent on dietary sources of such amino acids. Because no single seed type contains a complete regime of essential amino acids (cereal seed proteins are deficient in Lys, and legume seed proteins are mostly deficient in the sulfur amino acids, Met and Cys), genetic manipulations have been used in attempts to improve essential amino acid balance (157). However, negative effects on agronomic traits, such as germination, were observed in this study. Thus, by enhancing Lys synthesis and blocking its catabolism, elevation of Lys levels in *Arabidopsis* seeds causes a retardation of seed germination (7). In this latter study, metabolome and transcriptome analyses revealed a negative impact of Lys accumulation on tricarboxylic acid (TCA) cycle activity, and an attenuation of the boost of specific transcriptional programs that are essential for seedling establishment, such as the onset of photosynthesis or the turnover of specific transcriptional programs associated with seed embryonic traits (7). Therefore, catabolism of the Asp family of amino acids is an important contributor to the energy status of plants, and hence to the onset of autotrophic growth-associated processes during germination (7).

Ethylene AdoMet is also the precursor of ethylene, which regulates seed germination by promoting the weakening and rupture of seed tissues surrounding and enclosing the radicle and by counteracting the inhibitory action of ABA on these processes (89). There is evidence in support of interaction between ethylene, ABA, cytokinin, and ROS signaling in controlling seed germination and early seedling development (9, 112, 137) (figure 3).

Altogether, these data support the model that the Met cycle behaves as a hub to control metabolic activity in germinating seeds in concert with the action of the principal hormones and signaling molecules involved in regulation of seed germination and seedling establishment (figure 3).

Compartmentalization of metabolism

Studies in different plant species have documented that the seed transcriptome and proteome exhibit tissue-specific features (26, 51, 80, 102, 104). For example, in Lepidium sativum (a close *Arabidopsis* relative with larger seeds), tissues express different sets of genes during germination. These differential gene expression levels between tissues relates to their cognate functions, i.e., the radicle as a growing tissue and the micropylar endosperm cap surrounding it as a regulator of germination through weakening (104). Also, *Medicago truncatula* (51) and sugar beet (26) seeds show compartmentalization of metabolic activity between the various seed tissues (e.g., sulfur, oxalate, or phytate metabolism), indicating a division of metabolic tasks between these tissues.

At the subcellular level, transcriptome profiling in rice seed germination has revealed that for the mitochondrial gene set, a greater proportion of transcripts peaks early, at 1 or 3 HAI, compared with the plastid set; notably, many of these transcripts encode proteins involved in transport functions. By 24 HAI, components of the mitochondrial import apparatus decrease in abundance by at least tenfold, and components involved in metabolism increase in abundance

by at least tenfold (62). This increase in transcripts encoding proteins involved in energy is also observed for the plastid gene set, which correlates with the requirement for large amounts of energy in early stages of germination.

In *Arabidopsis*, nuclear-encoded transcripts for plastid-localized proteins are induced between 6 and 12 HAI during seed germination. This induction is repressed by ABA, suggesting a role of these genes in the maintenance of dormancy and of embryonic identity (13). Transcription of the plastidial genome has been implicated in the regulation of germination potential, because *Arabidopsis* seeds imbibed in Tagetin, an inhibitor of the plastid-encoded RNA polymerase, show a delay in seed germination (38). Consistent with the rice data (62), the *Arabidopsis* plastid may be regulating germination potential through the production of energy. The upregulation of the photosynthetic machinery may also be a reflection of the seed's commitment to germinate in anticipation of autotrophic growth (13).

Also, in-depth temporal transcriptome profiling revealed germination-specific genes in *Arabidopsis*. This group of genes, which is enriched in genes encoding mitochondrial proteins, highlights the crucial role of mitochondria for successful germination (107).

Seed vigor

Manipulating seed vigor

Seed vigor is a complex seed property that determines its potential for rapid uniform emergence and development under a wide range of field conditions. The life span of seeds is an important component of seed vigor, which depends on the seeds' physiological and genetic conservation potential and on conditions encountered during storage (116, 145). Lethal damage can be experimentally induced at a high rate in seeds by submitting them to controlled deterioration treatments, which mimic natural aging (37, 119). This treatment, which involves the elevation of seed moisture content and temperature, is frequently used to quickly assess seed quality. Seed vigor can also be enhanced by treatments referred to as seed priming, which have proved successful at invigorating the performance of low-vigor seeds (21, 58, 72, 98). In this case, seeds are subjected to a controlled hydration, so as to initiate germination-related processes while preventing radicle emergence. In most plant species, seeds can remain desiccation tolerant up to radicle emergence; therefore, priming can be followed by a dehydration step permitting storage of the primed seeds (figure 4). Despite the wide use of these treatments, their optimization currently rests on carrying out germination assays, which can provide only retrospective indications of the effectiveness of the priming conditions.

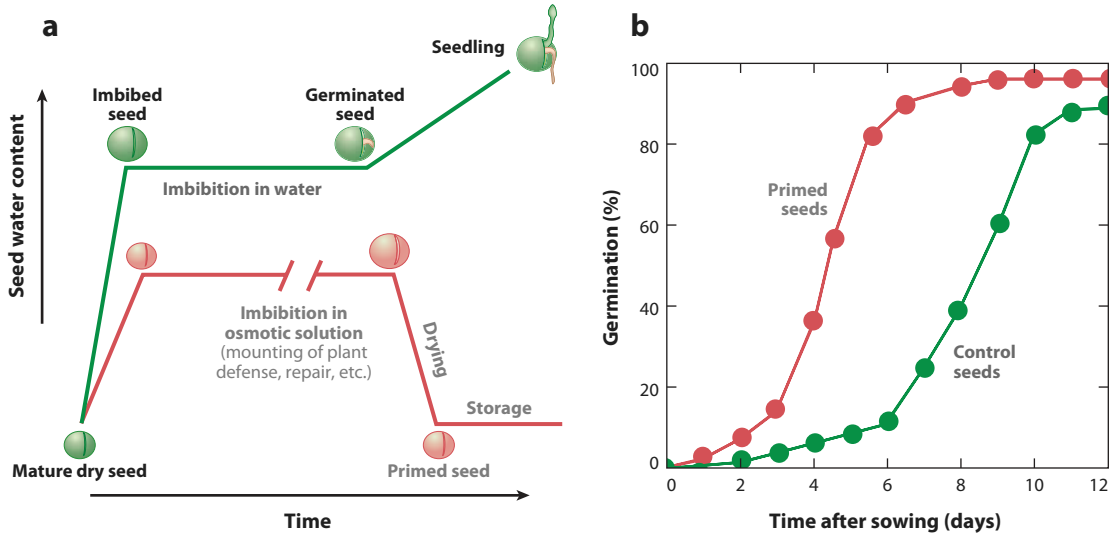

Figure 4: Seed priming. Seed priming as a presowing seed treatment can alleviate the adverse effects of environmental stress on germination performance. (a) Seed water relations during priming. Seed priming consists in partial hydration of the seed to a point where germination processes are begun but not completed. Most priming treatments involve imbibing seeds with restricted amounts of water (e.g., in osmotic solutions, referred to as osmopriming) to allow sufficient hydration and advancement of metabolic and repair processes while preventing germination or loss of desiccation tolerance. For storage purposes, treated seeds are redried before use. (b) Improved germination vigor of primed seeds. Sugar beet seeds submitted to osmopriming as described (primed seeds) exhibit rapid germination in comparison with untreated seeds (control seeds) when reimbibed under normal or stress conditions (72). Germination experiments were conducted at 5°C. Adapted from C. Job, J. Catusse & D. Job, unpublished data.

To unravel biomarkers of seed vigor, a comparative proteomic study was conducted with sugar beet seed samples of varying vigor as generated by priming and controlled deterioration treatments (25). Seeds were also submitted first to controlled deterioration and then to priming to evidence reversible changes in protein accumulation patterns. Specific signatures were shown to correlate with seed vigor, suggesting their role in this trait. These signatures include the sulfur amino acid pathway as well as several metabolic pathways involved in lipid and starch mobilization, protein synthesis (translation initiation factors), components of ABA signaling pathways, or the methyl cycle.

As mentioned above, when a germinating seed encounters osmotic stress, its growth is inhibited and embryonic programs are reinitiated in response to ABA, thus preventing the plant from precociously entering the vulnerable seedling state (92, 93) (figure 1). Because seeds experience osmotic stress in the limiting water conditions used in priming treatments, it is tempting to propose that priming increases seed vigor not only by initiating germinationrelated processes but also by allowing the growth-arrested seeds to reinforce their capacity to mount adaptive defense responses useful to withstand environmental stress during seedling establishment. This could occur thanks to the stored proteins and/or mRNAs that have been shown to function prior to radicle protrusion in seed imbibition (79, 117). Consistent with this, the stability of stored mRNAs was shown to be an important determinant of seed vigor (129, 132). Early repair mechanisms are the most probable explanation for the beneficial effects of priming treatments (128).

The characterization of three genes (NnMT2a, NnMT2b, and NnMT3) from sacred lotus that encode metallothioneins, which are cysteine-rich small proteins involved in ROS scavenging, was recently reported, and their roles in seed germination vigor were evaluated. These genes were highly expressed in germinating sacred lotus seeds and were dramatically upregulated in response to high salinity and oxidative stresses. Moreover, transgenic *Arabidopsis* seeds overexpressing NnMT2a and NnMT3 displayed a remarkably improved resistance to accelerated aging treatment, indicating their significant roles in seed germination vigor. Taken together, these data demonstrate that overexpression of NnMT2a and NnMT3 in *Arabidopsis* significantly enhances seed germination vigor under abiotic stresses, presumably by improving antioxidant activity at an early developmental stage (156).

In the field, Seeds Can Undergo Wet-Dry Cycling

Seed survival in the soil contributes to population persistence and community diversity. In contrast to the long-standing view that dry storage is necessary to ensure seed longevity, seeds in nature, which are buried in the soil, may experience wet-dry cycling that is akin to the wellstudied commercial process of seed priming in which seeds are hydrated and then redried to standardize their germination characteristics. In other words, an incomplete hydration would allow seed cellular activity to be restarted without permitting radicle protrusion. As seeds will keep their tolerance to desiccation under these conditions, they could survive a subsequent drying event.

The influence of such wet-dry cycling has been studied at the level of seed persistence (defined as in situ longevity) for the global agronomic weed Avena sterilis ssp. ludoviciana. fieldaged seeds that underwent numerous wet-dry cycles due to natural rainfall maintained high viability, correlated with resynthesis of protective antioxidant compounds such as GSH (91). Wet-dry cycling may also affect the dormancy level of buried seeds (15) and is surmised to allow the activation of repair processes such as the repair of damaged DNA, proteins, membranes, and mitochondria via stored mRNAs and stored proteins (82). It is noted that seeds exhibiting exceptional longevity have been collected in soil (e.g., beneath rubble; 126).

Concluding remarks

Postgenomics studies have provided important new information about mechanisms controlling germination. Global molecular profiling can be monitored at the three levels of gene expression, transcripts, proteins, and metabolites. Specific expression signatures are observed as soon as 15 min after imbibition. The data are consistent with a strong preparation of germination during seed maturation on the mother plant prior to seed dispersal and with a combined use of stored proteins, stored mRNAs, and de novo transcription to achieve successful seedling establishment. A major control seems to be exerted at the translational level. The metabolism of Met is central to the control of seed germination, and seems to constitute a hub for germination regulation. Besides its prime importance in housekeeping metabolic pathways, this metabolism is linked to several hormone pathways that are well known to regulate germination, thus illustrating for the first time a link between metabolic control and hormonal regulation of seed germination.

Currently, "-omics" approaches are being used to characterize seed vigor, which encompasses seed longevity, tolerance of germination to environmental stresses, and the uniformity and speed of seed germination and seedling establishment. These studies are expected to deliver new markers of seed quality that can be used in breeding programs and/or in biotechnological approaches to improve crop yields.

Summary points

1. Orthodox seeds of higher plants represent highly sophisticated biological systems that, through intense desiccation, can interrupt their development on the mother plant and restart their cellular activity following imbibition.
2. The maturation program determines a substantial part of the seed germination process. Specific mRNAs and proteins are stored in the mature seed to prepare for reactivation of cellular activity following imbibition.
3. Prior to radicle emergence, seeds maintain a flexible and reversible status: Whenever the conditions for seedling growth are not met following initial imbibition, seeds can reverse their program and recapitulate the maturation process.

4. De novo transcription is not mandatory for early stages of germination but is necessary for regulation of the germination rate and for seedling establishment.
5. Comparison of dry seed versus germinating and germinated seed transcriptomes and proteomes indicates regulation at transcriptional, posttranscriptional, translational, and posttranslational levels.
6. Beyond ABA and GAs, which are involved (respectively) in the early and late stage of the germination process, other phytohormones (ethylene, brassinosteroids, salicylic acid, cytokinin, auxin, jasmonic acid, oxylipins) and radicals (reactive oxygen and nitrogen species) play a key role in seed germination.
7. The sulfur amino acid metabolism is instrumental to the physiology of seed germination, allowing the linking of housekeeping metabolic activity and hormonal regulation.
8. To increase crop yields, biomarkers of seed vigor can be revealed by genetic approaches and seed treatments.

Future issues

1. finely identifying and characterizing transcripts (stored and de novo synthesized) and stored proteins, whose action is required following imbibition, as well as the mechanisms underlying their mobilization and regulation, will help provide a better understanding of the preparation of the germination process during seed maturation on the mother plant and the transitions needed in gene expression for commitment to productive germination and seedling establishment following imbibition. At the practical level, this knowledge is important to better predict harvest dates corresponding to maximal seed quality.
2. Further developing a systems approach to the germination process and seed vigor will greatly help in unraveling the principal biochemical and molecular mechanisms controlling such complex traits that are unique to plants.
3. The characterization of biomarkers of seed vigor for seed improvement via breeding programs and/or technological and biotechnological approaches will allow the production of seeds of the highest possible quality with the goal of improving crop yields, particularly under stressful environmental conditions. This is particularly acute in the context of climate change and an increasing world population.
4. Deciphering the biochemical and molecular mechanisms occurring during wet-dry cycling of seeds buried in soil is of paramount fundamental and ecological importance to characterize features accounting for the exceptional longevity of seeds once liberated in the environment from the mother plant.

Disclosure statement

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Acronyms

ABA abscisic acid
AdoHcy *S*-adenosylhomocysteine
AdoMet *S*-adenosylmethionine
Cys cysteine
Cyst cystathionine
GA gibberellin
GSH glutathione
HAI hour after imbibition
Hcy homocysteine
KAPA 7-keto-8-aminopelargonic acid
LEA late embryogenesis abundant
Met methionine
OPH *O*-phosphohomoserine
PIMT protein L-isoaspartyl-*O*-methyltransferase
QTL quantitative trait locus
ROS reactive oxygen species
SMM *S*-methylmethionine
Thr threonine
Trx thioredoxin